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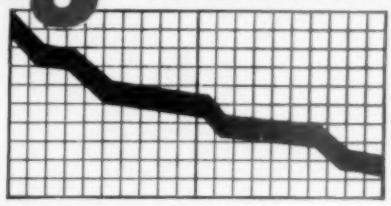

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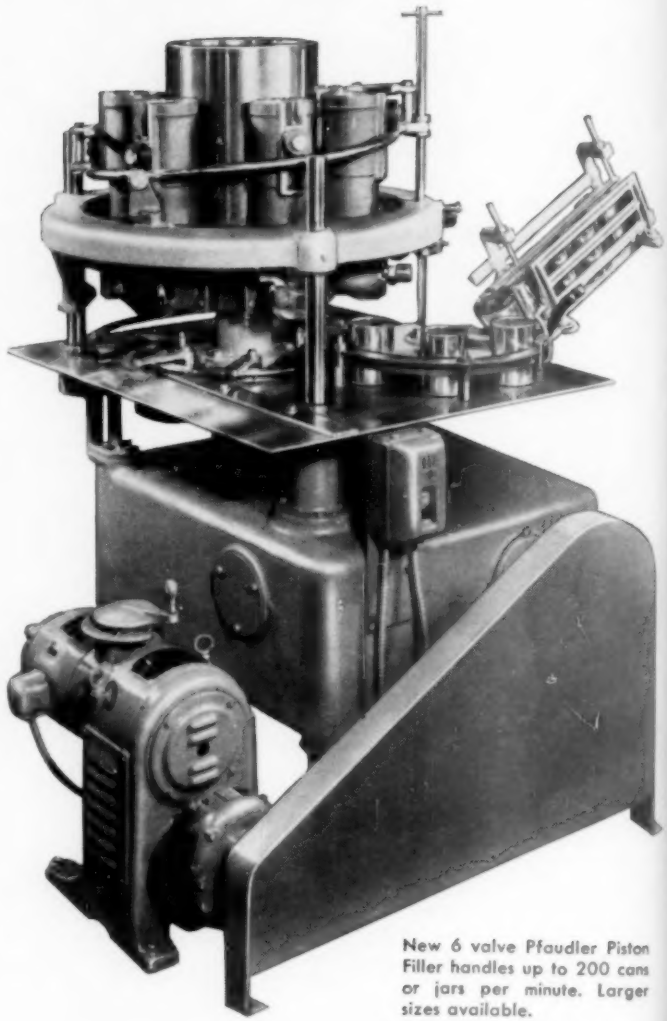

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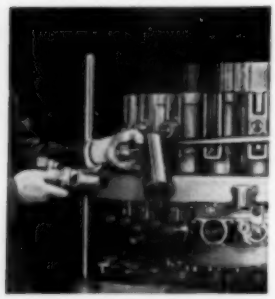

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Factors Influencing the Quality of Tomato Products. I. Surface-Localized Pectic Enzymes Inactivated by Blanching

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(Manuscript received October 19, 1951)

Pectic enzymes in ripe Pearson and San Marzano variety tomatoes occur in greatest quantity near the surface of the fruit; therefore a large proportion of such enzyme activity may be destroyed prior to crushing by passing whole fruit through a steam or hot water blanch. Such treatment can improve the retention of pectic substances during processing and tomato products of higher consistency may be obtained.

It is well known that enzymic destruction of pectic substances during processing is one of the factors contributing to low consistency in tomato products *(2, 6, 7)*. This destruction can be greatly reduced by rapidly heating freshly crushed tomato macerate to 185° F. (85° C.). However, the action of these enzymes is very rapid *(4)* and it has been found in the course of earlier work in this laboratory *(6)* that 100% retention of pectic substances is not obtained even under the best practical commercial conditions of rapidly preheating freshly crushed tomato macerate. This is due to the fact that heating and inactivation of pectic enzymes cannot be made instantaneous and therefore a finite time interval exists during which some destruction of pectic substances will occur. Increased retention of pectic substances, with resulting improvement of consistency in tomato products, might be possible if some practical means were found to inactivate a large percentage of the pectic enzymes prior to crushing and preheating.

British workers have reported *(8)* that oxidizing enzymes in the tomato fruit are located largely in the skin and outer portions. If pectic enzymes are similarly located, they might be inactivated by scalding in steam or hot water prior to crushing. This heat treatment would probably reduce the degradation of pectic substances in the short interval between crushing and attaining the proper preheating temperature of 185° F. (85° C.)

This paper reports investigations of the location of pectic enzymes in ripe tomato fruit and application of an improved processing method whereby greater retention of pectic substances is attained.

EXPERIMENTAL

Location of pectic enzymes. The tomato fruit has been described *(1)* as consisting of an outer wall (pericarp) the radial walls, the core, and the locular cavities containing the seeds embedded in a jelly-like parenchyma tissue originating from the placenta. The pericarp, in turn, is described as consisting of 3 distinct sections: the epidermis, a single layer of cutinized cells; the epicarp, consisting of 3 or 4 layers of collenchyma cells; and the mesocarp, consisting of parenchyma tissue which constitutes the bulk of the pericarp.

Whole ripe tomato fruit (Pearson var.) was dissected to separate these morphological sections. The fruit was cut in halves through the axis. One half was then separated as completely as possible into epidermis, epicarp, mesocarp, radial walls and core, and locular cavity contents. The weight of each was determined and the individual fractions were macerated in a micro-blender with an equal weight of 10% sodium chloride solution,[b] and filtered. Pectin-methylesterase (PM) and pectic acid-depolymerase (DP) were then determined in each extract *(6)*. The remaining half of the fruit was comminuted with an equal weight of 10% sodium chloride solution and PM and DP activities were determined on filtered extracts. The total pectic enzyme content of the whole fruit was calculated from these data and used in calculating the percentages of the total pectic enzyme activity of the fruit in each of the sections. In this manner, 25 ripe Pearson tomatoes were analyzed. The percentage distribution of pectic enzymes in the sections was averaged for all fruits. The data are presented in a condensed form in Table 1 together with the approximate thicknesses of the sections involved. In the same manner, 25 ripe San Marzano (pear-shape) tomatoes were analyzed with essentially the same results as given in Table 1, except that the thicknesses of morphological sections differed.

TABLE 1

Location of pectic enzymes in ripe Pearson tomatoes

Section of fruit	Approximate thickness of sections	Percent of total PM activity found in section[c]	Percent of total DP activity found in section
Epidermis plus epicarp	1/16 inch	25-50	25-50
Total pericarp including epidermis and epicarp	¼-½ inch	65	65
Radial walls and core	.....	35	35
Locular cavity	.....	0	0

[c] The tomatoes examined varied in absolute pectin-methylesterase units (PMU) content from 15 to 35 PMU per g. on a whole fruit basis. Although there is considerable variation, the total pericarp makes up approximately 45% of the weight of the whole fruit; the radial walls and core about 35%; and the locular cavity contents about 20%.

Discussion

In Table 1, the data for the epidermis and epicarp have been combined because of the uncertainty of perfect separation of the fractions by dissection and the high variability among individual fruits in the ratio of PM and DP in the 2 sections. For the same reasons no value is given for the mesocarp. This value was replaced by a value for the total pericarp, which includes epidermis, epicarp, and mesocarp. It is interesting to note that, in spite of variations in the percentage of pectic enzyme contents of these 3 fractions, the epicarp was always found to contain a higher percentage than either the epidermis or the mesocarp. This fact strongly indicates the localization of pectic enzymes near the fruit surface. It is also interesting to note that the distribution of the two enzymes was always related to differences in color and distribution of color. Thus the redder tomatoes were found to have a higher total pectic enzyme content than more pale tomatoes. In redder

[a] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[b] In contrast to experience with citrus fruit *(3)*, it was found that the above method of extraction applied to all types of tomato tissue yields the same amount of PM as extractions employing combinations of salt and elevated pH.

tomatoes there was a greater concentration of the red pigment (lycopene) in the epicarp, whereas in paler tomatoes, the distribution of pigment was more diffuse and this pattern was repeated by the pectic enzyme distribution. Even within one type of tissue, such as the radial wall, it was found that local differences in color were accompanied by similar differences in pectic enzyme content even to the extent that colorless sections of radial wall were practically free of these enzymes.

In spite of the above-mentioned differences, the ratio of pectic enzyme content between total pericarp and other major morphological sections was fairly constant.

Application of Blanching

To evaluate the effect of blanching on the retention of pectic substances and consistency, two 15-pound lots of Pearson variety tomatoes were processed into purees containing 21.5% solids.

Aside from the blanching of 1 lot (to be described later), the tomatoes were crushed and finished in one operation in a pilot-plant model tomato finisher having a screen with 0.033 inch perforations. The juice issuing from the finisher outlet was pumped rapidly through a heat exchange unit which brought the temperature up to 200° F. (93° C.) in about 1 second. The resulting juice from each preparation was then concentrated in a vacuum evaporator. The temperature of the juice during evaporation did not rise above 110° F. (43° C.). Concentration of the juice was discontinued when the solids content, determined refractometrically, reached 21.5%, since above this solids content it was difficult to get the concentrated material to circulate through the heat-exchanger.

One lot was blanched prior to preparation of the puree. Blanching was accomplished by immersing the tomatoes in hot water at 200° F. (93° C.) for exactly 2 minutes. The rate at which the heat exchanger could accept juice was limited and it was anticipated that blanching the whole lot at one time would be undesirable since an appreciable fraction would stand for some time after blanching before it could be fed through the crusher-finisher to the heat exchange unit. This would permit action of pectic enzymes at depths in the fruit where the temperature had become high enough to cause disruption of cell structure but not high enough to inactivate pectic enzymes (185° F. or 85° C.). Therefore, the tomatoes in this lot were blanched a few at a time as they could be accommodated. Even under these conditions, it is estimated that average hold-up time between blanching and preheating was 2 minutes. The temperature of the macerate during this holding time varied in the range of 122-140° F. (50-60° C.).

In the experiment where blanching was not employed, the whole tomatoes were fed to the crusher-finisher only as fast as the heat exchanger could accommodate the resulting juice, and the hold-up time between crushing and preheating was calculated to be only about 30 seconds.

The resulting purees were analyzed for pectic substances content *(6)*, and their consistencies determined using the Jacobs Gel-Time Viscosimeter with a 150 r.p.m. motor and the stainless steel tapered helix.[4] The scale readings of the instrument had been previously calibrated in centipose with standard Newtonian solutions of known viscosities and the consistencies of the puree samples determined at 30° C. (86° F.) are expressed in "apparent" centipoise viscosity corresponding to these calibrations. Since the viscous behavior of tomato puree is non-Newtonian, these values show only differences in consistency and do not represent absolute viscosities. This type of expression does have the advantage, however, of giving numerical values corresponding to the reproducible calibrations of the instrument.

[4] The mention of special instruments throughout this paper does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

Discussion

The results obtained are illustrated in Table 2. As was expected on the basis of distribution of pectic enzymes, blanching improved the retention of pectic substances during processing and the resulting puree had a higher consistency, which was also evident upon subjective observation. It is worth pointing out that both purees represent products with relatively high

TABLE 2

Pectin retention and consistency in tomato purees prepared with and without blanching

Sample	Solids[a]	Total pectic substances	Consistency "apparent viscosity"
	%	%	*cp.*
Puree from unblanched fruit	21.5	0.181	1,700
Puree from blanched fruit	21.5	0.281	1,850

[a] By refractive index.

retention of the pectic substances. The consistency data confirm previous findings *(6)* that, at relatively high contents of pectic substances, differences in consistency are not directly proportional to the amounts of pectic constituents present.

In evaluating the overall effect of blanching, some consideration must be given to the times and temperatures occurring during these pilot plant preparations. It has been noted that in the blanching experiment the tomatoes were held after blanching for about 2 minutes on the average before crushing and preheating was completed. As a result, there was time for some pectic enzyme action within the insufficiently heated portions of the fruit before crushing. In addition, the crushed macerate was at an elevated temperature [122-140° F. (50-60° C.)] at which enzyme activity is greatly accelerated *(5)* during the short interval between crushing and preheating. On the other hand, where blanching was not employed, enzyme activity was not initiated until the fruit was mechanically crushed and the rate of enzyme action would then be relatively slow in the cold macerate before preheating inactivated the enzymes. Therefore, except for the inactivation of pectic enzymes by blanching, conditions of the experiment favored the retention of pectic substances in the unblanched lot of fruit. The fact that a greater retention of pectic substances was nevertheless obtained in the blanching experiments lends even greater weight to the results presented and to the conclusions drawn.

The blanching operation, as applied here, or as sometimes employed by the industry for greater color extraction, inactivates only the surface-located pectic enzymes but at the same time it causes disruption of cells within the fruit where temperatures would not be sufficient to inactivate the enzymes. Little advantage in consistency would therefore be gained by blanching unless this were combined with a rapid and efficient preheating after crushing. This is particularly true since the activity of the residual pectic enzymes will be greatly accelerated at the higher temperatures pertaining in the resulting macerate from blanched tomatoes before it is preheated to 185° F. (85° C.). However, when employed in conjunction with previously recommended conditions of processing *(4)*, it appears that blanching will afford

significant advantage in pectic substances retention and improved consistency of the product.

Further studies are under way to determine optimum times and temperatures of blanching to study the suppression of pectic enzyme activity in tomatoes macerated cold.

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Peanut Butter. II. Effect of Roasting and Blanching on the Thiamine Content of Peanut Butter[a]

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(Manuscript received February 11, 1952)

The effects of roasting and blanching on the thiamine content of peanuts in the manufacture of peanut butter have been investigated. Most of the thiamine of the raw peanuts was found to be contained in the kernel after removal of the testa. Peanut butters made from peanuts roasted to various extents were found to contain only a relatively small proportion of the thiamine originally present in the kernel. Furthermore, with increase of roasting the amounts of thiamine found in peanut butter were progressively less, while the color of the product became darker. Consequently, color becomes a visual indication of the extent of roasting, and indirectly of the loss of thiamine.

Raw peanuts are a good source of thiamine (vitamin B_1) *(3, 6)*, but roasted peanuts are grouped by Booher and Hartzler with those foods which generally contain only a moderate amount *(3)* of this substance. Because of the known thermal instability *(7)* of thiamine and the high temperature and protracted time of heating peanuts to produce the desired color, flavor, and aroma in peanut butter *(9)*, it might be expected that there would be some decrease in the content of this vitamin in the finished product.

EXPERIMENTAL

Peanuts. The peanuts used in this investigation were No. 1 grade, shelled, white Spanish peanuts *(8)* obtained from a commercial shelling plant in the southeastern section of the United States.

Processing conditions. The peanuts were roasted in 2-bag (250 lb.) batches in a natural gas-fired, radiant heat roaster *(2)*. The roasted peanuts were cooled, mechanically blanched, and manually sorted to eliminate objectionable material. Details of the processing conditions have been reported previously *(9)*, and only sufficient information is given here to permit identification of the finished product. The blanched, sorted peanuts were ground into peanut butter at the rate of about 240 pounds per hour in an 8-inch, vertical attrition mill of a type commonly employed in the commercial manufacture of peanut butter. The temperatures of the peanut butters issuing from the mill varied from about 150-165° F. (65 to 74° C.).

Samples and method. Raw, shelled peanuts; raw, shelled peanuts from which the testa (red skin) had been removed manually; and the peanut butter made from roasted peanuts were analyzed for thiamine content. Results of analyses of raw, shelled peanuts before and after removal of testa, and similar data for the peanut butters are given in Table 1.

Thiamine was determined by a modification of the Conner and Straub *(5)* fluorescence method. The method is based on the oxidation of thiamine to thiochrome, which fluoresces in ultraviolet light. In the absence of other fluorescing materials, the fluorescence is proportional to the thiochrome present and hence to the thiamine content of the original sample. The procedure used is virtually the same as the thiochrome method of The Association of Vitamin Chemists *(1)*. The modifications were made to increase the sensitivity sufficiently to determine the small amounts of thiamine found in some of the products.

The major steps in the modified thiochrome method are as follows:

1. Acid extraction. Accurately weigh a sample expected to contain from 1 to 5 γ of thiamine, and extract with 65 ml. of sulphuric acid for 30 minutes.
2. Incubation. A 0.3-g. sample of Taka-diastase[f] in 5 ml. of 2.5 *N*. sodium acetate is added to the cooled acid-extract, and the sample is subjected to enzymatic hydrolysis for 3 hours at 45 to 50° C.
3. Chromatographic purification. The sample is purified by passing the solution through an 8 cm. column of activated Decalso[f] followed by elution with hot acid potassium chloride solution.

[a] Report of study made under the Research and Marketing Act of 1946.

[b] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[f] It is not the policy of the U. S. Department of Agriculture to recommend the products named above over those of any others manufactured for the same or similar purposes. Their names are furnished solely for the convenience and information of the reader in ascertaining complete details of the method of assay.

TABLE 1

Thiamine in raw peanuts and peanut butters

No.	Thiamine in raw shelled peanuts		Total thiamine contained in kernel of raw shelled peanuts [c]	Roasting time [d]	Roasting temperature		Color of roast [d]	Residual thiamine in peanut butter (as received)	
	Kernel testa: Kernal only (as received)				*Minimum*	Maximum			
	γ per gram	*γ per gram*	%	*minutes*	° *F.*	° *F.*		*γ per gram*	% [c]
1	.....	.....	.....	17	230	275	very light	4.04	.....
2	.....	.....	.....	23	205	283	very light	2.30	.....
3	13.6	10.98	78.8	21	205	285	light	2.41	21.9
4	14.10	12.56	86.9	22	200	285	light	2.07	16.5
5	12.48	10.28	80.3	21.5	205	285	light	2.00	19.5
6	12.71	11.89	91.2	25	200	292	light	2.12	17.8
7	12.45	11.16	87.3	22.5	200	290	medium	1.53	13.7
8	.....	.....	.....	21	210	290	medium	1.39	.....
9	17.15	14.05	79.9	26	200	298	medium	1.74	12.4
10	13.56	11.10	79.8	24	200	300	medium	1.40	12.6
11	9.66	8.66	87.4	23	215	295	dark	1.10	12.7
12	13.16	11.08	82.1	26	200	308	dark	0.78	7.0
13	12.91	11.55	87.2	25	205	300	dark	.....	.....
14	14.16	13.59	93.6	29	200	304	dark	1.35	9.9
15	10.68	9.57	87.4	24	210	300	dark	0.77	8.0
16	15.08	11.54	74.6	25	205	305	dark	0.74	6.3
17	12.22	10.63	84.8	20	240	305	dark	0.64	5.9
18	11.89	10.16	83.4	21	215	315	very dark	0.22	2.1
19	.....	.....	.....	17.75	250	320	very dark	0.15	.....
20	11.51	11.47	97.1	22.5	210	320	very dark	0.19	1.6

[c] Calculated on basis of kernel compressing 97.5% of the total weight of the raw, shelled peanut and the testa 2.5% of such weight.

[d] Detailed processing information has been reported for each sample in a previous publication *(9)*.

[e] Calculated on basis of thiamine content in the kernel of raw, shelled peanuts.

4. Oxidation. The sample, blank, and standard are oxidized as described in the procedure of The Association of Vitamin Chemists *(1)* for conversion of thiamine to thiochrome.
5. Photofluorometric measurements. The method *(4)* prescribed for use with a Coleman Model 12 Electronic Photofluorometer [f] including an appropriate filter to obtain maximum sensitivity is used instead of the procedure of The Association of Vitamin Chemists.
6. Calculations. Calculations are made as given in the method prescribed by the Coleman Electric Company *(4)*.

RESULTS AND DISCUSSION

The results of the analyses of raw shelled peanuts before and after removal of the testa (Table 1) show that from 74 to 97% of the total thiamine is concentrated in the kernel of the raw shelled peanut. Results of analyses of peanut butters (Table 1) made from peanuts roasted under different conditions *(9)* show that the content of thiamine diminishes with increase of roasting while the roasted peanuts become darker. Furthermore, comparison of the thiamine contents of peanut butters with those of the kernel in raw, shelled peanuts shows in general that butters made from light-roasted peanuts retain about 20% of the original thiamine content; those from medium-roasted peanuts less than 14%; those from dark-roasted peanuts less than 10%; and those from very dark-roasted peanuts less than 3%. Consequently, color becames a visual indication of the extent of roasting and, indirectly, of the loss of thiamine.

SUMMARY

An investigation was made of the losses occurring in the thiamine content of white, Spanish peanuts during the steps involved in the manufacture of peanut butter. The results show that most of the thiamine content (74 to 97%) of the raw, shelled peanuts is contained in the kernel after removal of the testa. It was also ascertained that a relatively small amount (2 to 22%) of the thiamine originally present in the kernel of the peanut remained after processing into peanut butter. Also, with increase of roasting the amounts of thiamine found in peanut butter were progressively less, while the color of the product became darker. Consequently, color becomes a visual indication of the extent of roasting, and indirectly, of the loss of thiamine.

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Atomization as a Factor Affecting Quality in Spray-Dried Albumen[a]

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(Manuscript received March 17, 1952)

A specially designed spray nozzle for the atomization of albumen is described. Experimental results are presented to indicate the effects of various atomizing schemes on the beating power of spray-dried egg white.

In a companion article the authors reported on the effects of shear and surface formation on the beating power of egg white *(1)*. In these studies it was shown that both of these forces may adversely affect this functional property. It was suggested that shear is of primary importance in atomizing egg white where conventional pressure nozzles and centrifugal atomizers are used. However, if rate of surface formation or total surface is excessive, these factors too may be expected adversely to affect beating power.

The present paper describes experiments designed specifically to study the effects of atomization on the beating power of albumen. Particular attention was directed towards evaluating certain atomizing schemes which were thought to minimize either or both the shear and the rate of surface formation.

EXPERIMENTAL

The egg white used in these studies was obtained from 1, shell eggs produced on the College Poultry Farm and 2, commercial, milled frozen whites. With the former the whites were separated from the yolks and mixed in a Waring blendor just sufficiently to effect a uniform, free-flowing liquid. The latter product was from the same source as used in the companion study. When desired, fermentations were carried out by inoculating the egg white with a culture of a special strain of *Aerobacter aerogenes* and incubating at 93° F. (34° C.) until reducing sugar-free.

Three atomizers were employed in these studies: 1, An external mixing, two-fluid nozzle (Type ¼ J, Setup 1A, Spraying Systems Co., Chicago), 2, an internal mixing, two-fluid nozzle (Sprayco size 4BM, Spray Engineering Co., Summerville, Mass.) and a special, external-mixing nozzle[4] shown in Figure 1. The latter was designed by one of the authors (DHB) for continuously producing and atomizing egg white foam. This device effects the mechanical mixing of egg white and air to produce free flowing foam; this foam is then atomized by an air jet.

The spray dryer used in these experiments is shown in Figure 2. The atomizer assembly was mounted on wheels, permitting rapid adjustments and changes; also with it atomized liquid could be collected outside the dryer. The air flow was approximately 6 feet per sec. Satisfactory drying occurred at inlet and outlet temperatures of 185-194° F. (85-90° C.) and 131-140° F. (55-60° C.), respectively. No evidence of heat damage in the dried products was noted under these conditions.

Particle size of atomized liquid was determined on samples collected on a microscope slide lightly coated with castor oil. Figure 3 shows the sampling device used for this purpose. As the cylinder is rotated the lengthwise slit comes into line with the spray stream; this permits the atomized particles to enter and come in contact with the coated slide. Replicate particle counts were made at 100× until constant average values were achieved. Particle size counts on dried material were made on

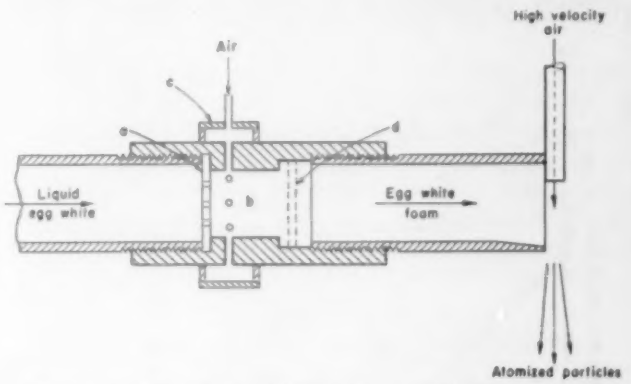

Figure 1. Special atomizing nozzle. a, liquid breakup orifices; b, liquid-air mixing chamber; c, annular air supply chamber; and d, mixing screens.

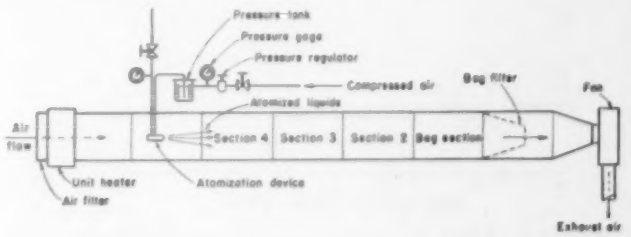

Figure 2. Laboratory spray dryer. The unit consists of a six-section horizontal, cylindrical chamber 22 inches in diameter and approximately 20 feet long. The fan located at the exhaust end pulls air through the chamber at a velocity of 5 to 6 feet per second. The powder collects at the bottom of sections 2, 3, and 4 and in the bag filter at the end of the last section.

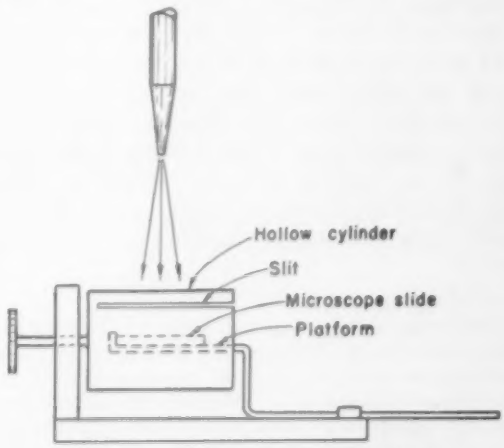

Figure 3. Atomized liquid sampler.

[a] Journal Paper No. J-2079 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 970.

[b] Present address: Henningsen, Inc., Sedalia, Mo.

[c] Present address: University of California, Davis, Calif.

[4] Patent applied for by authors.

very thin layers of powder spread on a slide coated with castor oil. The counts were made and averaged the same as with atomized liquid.

The various samples of atomized and/or dried albumen were finally evaluated using the meringue test described by Slosberg, Hanson, Stewart and Lowe *(4)*. Dried products were reconstituted to 12.5% solids before testing.

RESULTS AND DISCUSSION

The two-fluid nozzles were selected for test because it has been claimed that, in operation, they produce less shear than pressure nozzles and centrifugal atomizers *(3)*. The units were studied over a wide range of operating conditions. Both atomized liquid and spray dried powder were collected and evaluated.

The results obtained with two of these nozzles are shown in Figures 4 and 5 and Tables 1 and 2.[e] These

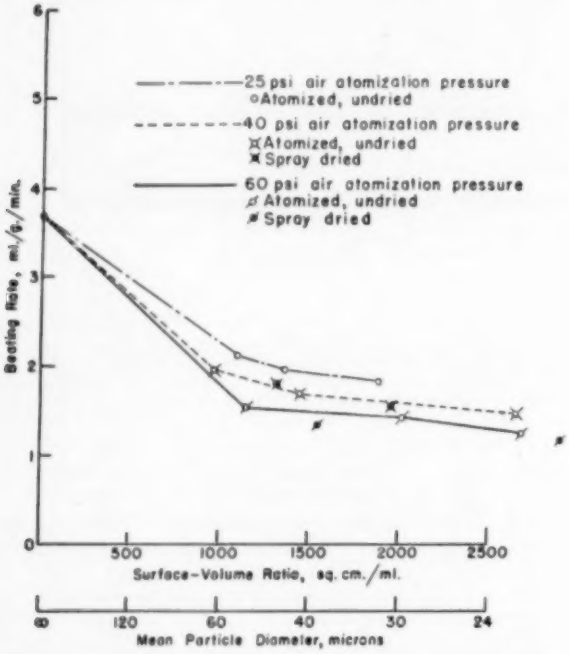

Figure 4. Effect of atomization and spray drying on beating power of egg white (internal mixing nozzle).

data show that all of the conditions of atomization tested caused severe losses in beating power. The damage occurred during atomization; drying caused no additional change. The degree of damage appears to be a function of particle size, air pressure (in the atomization nozzles) and nozzle design. It appears likely that the effects of the latter two factors are due to shear. In addition, mixing prior to final atomization might be expected to produce more shear than simple external mixing. In a separate trial simply forcing albumen through the internal mixing nozzle at 50 lbs./sq. in. without mixing with air produced no effect on beating power.

[e] Beating rate has been found to be an excellent measure of egg white performance. With the test described here, values of approximately 4 (ml. foam per g. of liquid per minute) indicate that the albumen under test is of excellent quality; it would make a high quality angel food cake. Values below 3.0 would signify that the albumen under test has suffered a significant loss in quality.

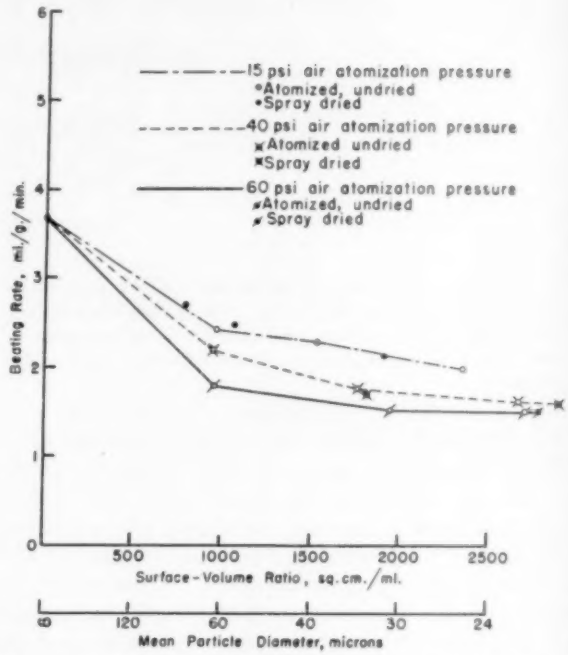

Figure 5. Effect of atomization and spray drying on beating power of egg white (external mixing nozzle).

Results of these and various other studies conducted in this laboratory suggested that an external-mixing, two-fluid nozzle of the type described by Hunkel *(2)* should help to minimize damage due to shear. In addition, a lowered rate of surface formation should help. A consideration of these points led to the development of the atomizing nozzle shown in Figure 1. This device permitted production of surfaces in two stages and atomization under relatively mild conditions.

Table 3 shows the effect of various operating conditions with this and other nozzles on the atomized al-

TABLE 1

Effect of atomization and spray drying on beating power of egg white [g]

Liquid pressure	Atomizing air pressure	Collection	Mean diameter of collected particles	Surface-volume ratio	Beating rate
		Atomized, undried			
Control	*p.s.i.g.*	....	*microns*	*sq. cm. per ml.*	*ml. per g. per minute* 3.65
1 p.s.i.g.	60	Entire spray	22.2	2700	1.51
10 p.s.i.g.	60	Entire spray	30.0	1940	1.51
30 p.s.i.g.	60	Entire spray	63.2	950	1.78
—7 inch water	40	Entire spray	22.6	2660	1.59
10 p.s.i.g.	40	Entire spray	34.3	1750	1.74
25 p.s.i.g.	40	Entire spray	64.0	940	2.18
—7 inch water	15	Entire spray	25.6	2340	1.96
7.5 p.s.i.g.	15	Entire spray	39.4	1520	2.24
20.0 p.s.i.g.	15	Entire spray	61.1	970	2.36
		Spray dried			
5 p.s.i.g.	60	Bag section	18.2	3300	1.50
10 p.s.i.g.	40	Bag section	20.7	2900	1.53
		2nd section	35.1	1710	1.66
14 p.s.i.g.	15	Bag section	31.5	1900	2.13
		2nd section	56.2	1070	2.46
		3rd section	75.0	800	2.70

[g] Commercial frozen egg white, *Aerobacter* fermented; external mixing nozzle.

TABLE 2

Effect of atomization and spray drying on beating power of egg white [h]

Liquid pressure	Atomizing air pressure	Collection	Mean diameter of collected particles	Surface-volume ratio	Beating rate
		Atomized, undried			
	p.s.i.g.		*microns*	*sq. cm. per ml.*	*ml. per g. per minute*
Control	....	....	....	....	3.65
1.5 p.s.i.g.	60	Entire spray	22.3	2690	1.25
5.0 p.s.i.g.	60	Entire spray	29.7	2020	1.45
10.0 p.s.i.g.	60	Entire spray	52.2	1150	1.57
—7 inch water	40	Entire spray	22.6	2650	1.45
2.5 p.s.i.g.	40	Entire spray	41.7	1440	1.66
5.0 p.s.i.g.	40	Entire spray	62.0	968	1.84
—7 inch water	25	Entire spray	31.7	1890	1.81
0.5 p.s.i.g.	25	Entire spray	44.0	1360	1.95
1.5 p.s.i.g.	25	Entire spray	54.7	1100	2.10
		Spray dried			
10 p.s.i.g.	60	Bag section	19.6	3060	1.11
		2nd section	39.4	1520	1.24
10 p.s.i.g.	40	Bag section	30.6	1960	1.57
		2nd section	45.4	1320	1.83

[h] Commercial frozen egg white, *Aerobacter* fermented; internal mixing nozzle.

TABLE 3

Effect of some selected atomization devices on beating power of egg white [i]

Type nozzle	Liquid pressure	Atomizing air pressure	Mean diameter of particles	Surface-volume ratio	Beating rate
		p.s.i.g.	*microns*	*sq. cm. per ml.*	*ml. per g. per minute*
Control	....	....	....	....	3.65
External	—26 inch water	50	25.6	2340	1.36
External	— 7 inch water	25	28.0	2140	1.44
External	24 inch water	20	39.3	1530	1.87
External	24 inch water	10	59.7	1000	2.28
Internal	1 p.s.i.g.	45	27.0	2220	0.94
Internal	1 p.s.i.g.	26	44.3	1355	1.22
Internal	1 p.s.i.g.	10	71.8	840	1.55
Special atomizer (10% stable foam)	4 p.s.i.g.	20	37.0	1620	2.30
	4 p.s.i.g.	13	57.7	1040	2.46
Special atomizer (16% stable foam)	5 p.s.i.g.	20	42.2	1420	2.49
	5 p.s.i.g.	13	52.4	1150	2.76
Special atomizer (32.5% stable foam)	5 p.s.i.g.	25	42.5	1410	2.87
	5 p.s.i.g.	20	52.8	1135	2.89

[i] Commercial frozen egg white, *Aerobacter* fermented.

bumen. Foams of varying stability [f] were atomized at several air pressures. It is obvious that in these cases the beating power was retained to a much greater degree than with the other two types of atomization; however, there is still a noticeable loss of beating power.

The experiments just reported were all carried out using commercial, milled frozen albumen. A few trials with fresh egg white showed a major difference in behavior. Some typical data on this point are presented in Table 4. These data clearly show that the commercial frozen product was far more susceptible to damage than the white from fresh shell eggs. As before, foam

[f] Foam stability is defined as the weight percent of albumen remaining as foam after holding 5 minutes. This value could be varied readily by controlling the ratio of air to albumen in the foam-producing section of the special atomizer.

TABLE 4

Effect of spray drying on beating power of egg white [j]

Source	Beating rate (ml. per g. per minute)			
	Control	External mixing nozzle [k]	Internal mixing nozzle [l]	Special atomizer [m]
Eggs, 2 days old	5.05	3.42	3.07	4.10
Eggs, 7 days old	5.60	1.95	1.73	2.81
Commercial frozen egg white	4.36	1.53	1.11	1.81

[j] Aerobacter fermented.

[k] Atomization conditions: 20 p.s.i.g. liquid pressure; 40 p.s.i.g. air atomization pressure.

[l] Atomization conditions: 10 p.s.i.g. liquid pressure; 60 p.s.i.g. air atomization pressure.

[m] Atomization conditions: 14 p.s.i.g. liquid pressure; 10-15 inch water air foaming pressure; 40 p.s.i.g. air atomization pressure.

atomization was the least destructive to beating power while the internal-mixing nozzle was the most detrimental.

Since egg white must be made reducing-sugar-free prior to drying to insure a stable product, all trials reported above were carried out using albumen fermented by using a special strain of *Aerobacter aerogenes*. Several trials were set up to determine whether this process affects the degree of damage due to atomization. Table 5 presents the results. It is obvious that fermentation

TABLE 5

Effect of spray drying on beating power of egg white

Source	Beating rate (ml. per g. per minute)			
	Control	External mixing nozzle [n]	Internal mixing nozzle [o]	Special atomizer [p]
Commercial frozen, unfermented	4.10	1.13	0.97	1.38
Commercial frozen, *Aerobacter* fermented	4.00	1.53	1.11	1.81
Commercial frozen, yeast fermented	4.48	1.12	1.00	1.66
Eggs, 7 days old, unfermented	4.36	2.05	1.42	2.78
Eggs, 7 days old, *Aerobacter* fermented	5.60	1.95	1.73	2.81

[n] Atomization conditions: 20 p.s.i.g. liquid pressure; 40 p.s.i.g. air atomization pressure.

[o] Atomization conditions: 10 p.s.i.g. liquid pressure; 60 p.si.g. air atomization pressure.

[p] Atomization conditions: 14 p.s.i.g. liquid pressure; 10-15 inch water air foaming pressure; 40 p.s.i.g. air atomization pressure.

did not much influence the degree of damage produced by atomization. If anything, fermented albumen was less affected than was the unfermented product.

SUMMARY

Results of these trials show that egg white is readily damaged by atomization. The damage is least in albumen from fresh eggs and greatest in the frozen, milled product. The effect was quite pronounced even where conditions of shear and rate of surface formation were minimized by special nozzle design.

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Radio Frequency as a Means of Grading Eggs[a,b]

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A method is described for measuring the radio-frequency conductivity of highly conducting liquids over a frequency range of 50 kc. to 1,000 mc. The conductivity of albumen from low-quality eggs was found to be 8.5×10^{-4} mho per cm. at 22° C. at the lower frequencies with increasing conductivity at the higher frequencies. Yolk material from low-quality eggs was found to have a conductivity of 2.9×10^{-4} mho per cm. at 22° C. at the lower frequencies. The conductivity of both albumen and yolk material from high-quality eggs was found to be about 15% lower than the respective values for low-quality eggs. Two electronic candlers using radio frequency were tested and found to be unsatisfactory. It was concluded that with the equipment now available, the interior quality of shell eggs could not be correctly measured with radio frequency.

Radio frequency has previously been proposed as a tool for determining egg quality *(4)*. Theoretically, it would be possible to determine egg quality by the use of radio frequency if the conductivity of the components (albumen and yolk) of high-quality eggs is substantially different than the conductivity of the components of low-quality eggs. Data concerning the specific conductivity of egg components at 1,000 cycles per second are available *(6)*, but the conductivity at higher frequencies has been measured on a relative basis only *(5)*. This report describes the instrumentation and method of measurement employed for determining the conductivity of components of high and low-quality eggs over a frequency range of 50 kilocycles to 1,000 megacycles per second. The problem of measuring the interior quality of shell eggs by the use of radio-frequency energy is discussed on the basis of the measurements made on the egg components. In addition, this report includes the results of tests on 2 devices reported to determine egg quality by a radio-frequency measurement.

INSTRUMENTATION

To simplify the instrumentation, a frequency range of 50 kc. to 1,000 mc. was selected for study. It was felt that measurements below 50 kc. would not be necessary, because the conductivity values should be the same as those reported in the literature *(6)* for 1,000 c.p.s. Measurements above 1,000 mc. are likely to be of little value, because the difficulty of generating and measuring these high frequencies indicates that it would be impractical to use this means for measuring egg quality. Instrumentation to cover the desired range of frequencies can be quite varied. The Boonton 160 A Q-Meter was chosen to cover the range from 50 kc. to 50 mc., and the Kay Electric Company U-H-F Mega-Match for the range from 50 mc. to 1,000 mc. At the time these measurements were made these 2 instruments were considered to be the best choice. Recently, however, better instruments have been devised for making impedance measurements at the higher frequencies. The newly-developed U-H-F Admittance Meter *(8)*, with its necessary accessories, would be superior to the instrument used for these particular studies.

For measurements at the higher frequencies, careful attention must be given to the type of cell to be used, in order to reduce errors from fringing effects, radiation and pick-up. A coaxial cell is best suited to conductivity measurements at the high frequencies, because the outer electrode provides almost a complete shield around the inner electrode thus preventing radiation and pick-up. For the measurements reported in this paper, a coaxial cell similar to that described by Brown *(2)* was constructed using aluminum for the electrodes and polystyrene for the insulator. The size of the cell is determined by the amount of material available from 1 egg and the impedance necessary to give the greatest accuracy of measurement. It was found by experimentation that a coaxial cell 3 cm. long with an outer electrode diameter of 3.50 cm. and an inner electrode diameter of 1.50 cm. would handle most cases, and that the other cases could be handled by using an inner electrode diameter of 2.50 cm. with the same outer electrode as before. Figure 1 shows a sketch

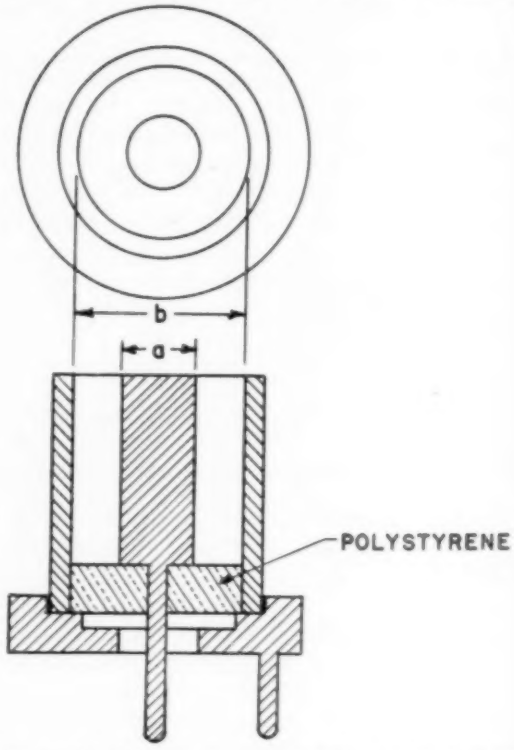

Figure 1. Coaxial cell: a, 1.50 or 2.50 cm.; b, 3.50 cm.

of the cell. Several identical cells of the above description were used, so that components could be compared without emptying and refilling the cells.

Since the cells, when filled with 10 ml. of egg component, have an impedance of about 10 ohms, the direct method of measurement cannot be used on the Q-Meter. In the direct method, the Q-Meter measures the Q (reactance divided by the resistance) of an unknown circuit element by introducing a known voltage into a tuned circuit incorporating this element and measuring the step-up of voltage that occurs in the tuned circuit. Since the voltage step-up is proportional to the Q of the circuit, the voltmeter can be calibrated directly in terms of Q. Impedances that cannot be resonated to form a high-Q circuit cannot be measured directly, but they can be measured by

[a] This paper was presented at the annual meeting of American Society of Agricultural Engineers at Houston, Texas, June 1951.

[b] Report of a study made with funds from the Research and Marketing Act of 1946.

inserting the unknown in series or parallel with a high-Q inductance, depending upon whether the unknown has a low or high impedance. The cells, representing low impedances, require the use of the series method in which the unknown is inserted in series with the inductance at the low-potential end of the coil and Q readings made with the unknown shorted out and with the unknown in the circuit.

The unknown resistance can be calculated from these measurements using the equation:

$$R = [1.59 \times 10^6 (C_1Q_1/C_2 - Q_2)/fC_1Q_1Q_2] \text{ ohms} \quad (1)$$

The conductivity of the component in the cell can then be computed from:

$$\sigma = [\log b/a \ (b^2 - a^2)/8RV] \text{ mho per cm.} \quad (2)$$

where R is the resistance of unknown in ohms; C_1 is the capacity setting in mmf. of Q-Meter with unknown shorted out; C_2 is the capacity setting in mmf. of Q-Meter with unknown in the circuit; Q_1 is the Q of circuit without unknown; Q_2 is the Q of circuit with unknown; f is the frequency in kc.; σ is the conductivity of component in mho per cm.; b is the diameter of outer electrode of cell in cm.; a is the diameter of inner electrode of cell in cm.; and V is the volume of component in ml.

The dielectric constant can generally be computed from the above measurements using the relationship,

$$X_c = [1.59 \times 10^6 (C_1 - C_2)/fC_1C_2] \text{ ohms} \quad (3)$$

to determine the reactive impedance. However, on the measurements with the egg components it was found that the reactive impedance was so small that it could not be determined. Therefore, no attempt was made to determine the dielectric constant.

To improve the acuracy over that which can be obtained with the Q-Meter alone, an auxiliary VTVM (Hewlett-Packard 410A) was used to measure the voltage across the resonant circuit. By switching range scales on this VTVM more appropriate voltage scales were available than on the single-range voltmeter of the Q-Meter. With the use of this arrangement, it was possible to obtain conductivity measurements with an accuracy of ± 5%.

The U-H-F Mega-Match, used above 50 mc., measures unknown impedances by determining the effect of the unknown on a transmission line of known characteristics. A pulse of high-frequency energy is sent into the transmission line, and is reflected from the end. With the use of a constant length of transmission line and a constant signal pulse, the reflected voltage depends on the impedance of the termination of the line. By comparing the voltage reflection from an unknown termination with the reflection of a short-circuit or open-circuit termination the impedance of the unknown can be computed by using the Smith chart *(7)*. Two factors need to be measured for the computation. The first is the reflection coefficient, or the ratio of the reflected voltage with the unknown termination to the voltage open circuited or short circuited; the second is the phase difference between the 2 reflected signals. An oscilloscope is best suited for making these measurements, and the requirements are a stable oscilloscope, preferably with a built-in voltage calibrator.

The measurements with the Mega-Match are subject to considerable error, because small irregularities in the transmission line produce unwanted reflections, and the phase angle cannot be accurately determined. In general, however, conductivities can be determined with an accuracy of ± 20% at the lower frequencies and ± 50% at the higher frequencies.

Because of the difficulty of controlling the temperature of the egg component, a small-size temperature-measuring element was inserted in the cell, and the temperature was observed during the measurements. A bead-type thermistor was found to be ideal for this measurement because of its small size, approximately 3 mm. diameter, and because it is insulated by a glass rod. Such a thermistor was used to measure the temperature by using a resistance bridge as a measuring unit.

The eggs for these studies were infertile eggs from Rhode Island Reds and White Leghorns. The high-quality eggs used were freshly laid while the low-quality eggs used had been allowed to deteriorate at room temperature. The eggs were broken out on a glass plate and given a quality score by using the USDA Chart *(1)* as a reference standard. A measured amount of the desired component was drawn by pipette from the egg and placed in the coaxial cell for measurement. After completion of a series of measurements the component was emptied from the cell and the cell thoroughly washed and dried before being used again.

Preliminary Studies

Tests using varying amounts of sample to determine the error caused by surface tension and fringing effects on the coaxial cell gave widely varying results for samples smaller than 3 ml. However, sample sizes from 4 to 12 ml. of component from a given egg showed conductivity variations of less than 5%, and since this is the limiting accuracy of the instrumentation, surface tension and fringing effects were considered to be negligible on samples larger than 4 ml. For the measurements reported in this paper at least 5 ml. of sample was used in all cases.

Since the coaxial cells containing the egg components were not covered, it was thought that errors might be introduced as a result of evaporation. However, repeated measurements on a given sample showed less than 5% variation in the conductivity for the first 4 hours. After 4 hours time the conductivity did change considerably, so all of the measurements were taken within 3 hours after opening the eggs to eliminate any error from this cause. Since the measurements are quite time consuming, several eggs were used to cover the complete frequency range under consideration.

Differences in temperature were found to be a source of considerable variation and measurements were made to determine the temperature sensitivity. Figure 2 shows the relationship of

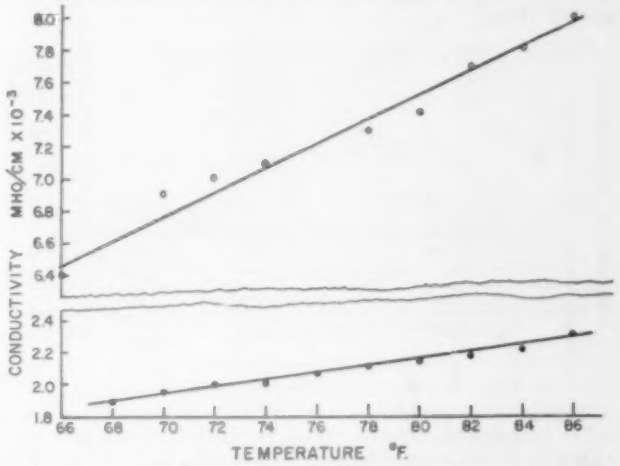

Figure 2. Conductivity variations with temperature for albumen (○) and yolk (●) of high-quality eggs at 800 kc.

conductivity to temperature for egg albumen and yolk. These curves show that the relationship is essentially linear and that the conductivity of each component increases about 1% for each degree F. of temperature increase.

RESULTS

The conductivity variations with frequency for low-quality (USDA score 10, 11, or 12) egg components at 72° F. (22° C.) are shown in Figure 3. The conductivity values of 8.5×10^{-3} mho per cm. for albumen and 2.9×10^{-3} mho per cm. for yolk at the lower frequencies agree well with the values of 8.7 and 3.1 reported by Romanoff and Grover *(6)* for 1,000 c.p.s. at a temperature of 25° C. The conductivity of each component was found to be constant up to frequencies of about 50 mc. Above this frequency the conductivity increased rapidly, and no peak was reached within the frequency range of the instruments used.

Figure 4 shows the variations of conductivity with frequency for high-quality (USDA score 1, 2, or 3) egg

components at 72° F. (22° C.). The conductivity values were again essentially constant for frequencies up to about 50 mc. and above this frequency conductivity increased rapidly. By neglecting the higher frequencies where the accuracy of measurement is very poor it was found that the components of high-quality eggs had about 15% lower conductivity than the low-quality components. Figure 5 shows the same curves from the previous two graphs on a single sheet for comparing the high-and low-quality values.

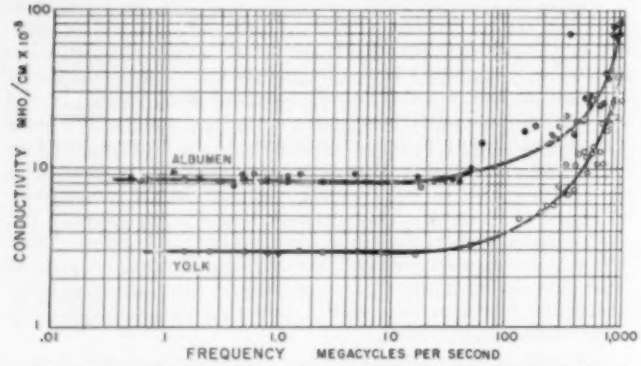

Figure 3. Conductivity of "C"-quality egg components at radio frequencies. Points represent individual measurements, although several eggs were used to cover the complete frequency range.

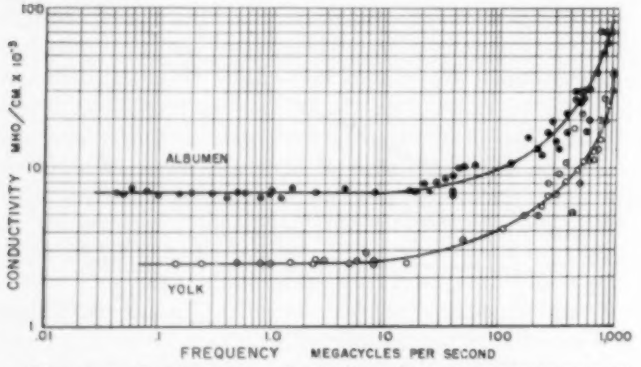

Figure 4. Conductivity of "AA"-quality egg components at radio frequencies. Points represent individual measurements, although several eggs were used to cover the complete frequency range.

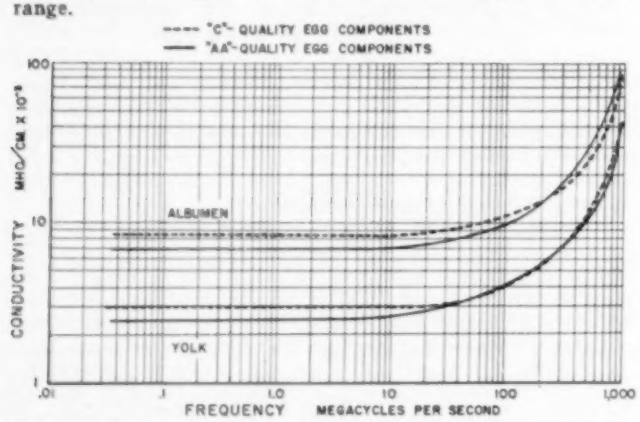

Figure 5. Conductivity of egg components at radio frequencies.

DISCUSSION

With only a 15% difference of conductivity between high-quality and low-quality components it is obvious that very accurate radio-frequency measurements are required to show differences between egg components that are related to quality. To be of practical value, a radio-frequency instrument for determining egg quality would be required to make measurements on intact eggs. Many variables which were controlled in the foregoing studies would thus be introduced, and these variables greatly affect the results. Variations in volume of test material would be introduced for two reasons. First, eggs vary in size; second, the amount of material contained in an egg varies because the shell is porous and evaporation takes place. This latter characteristic is recognized in egg trade channels as an increase in air-cell size. Unless fully compensated for, a larger egg would absorb more energy due to size alone, just as would an egg with a small air cell, because of the presence of a greater volume of material than in an egg of similar shell dimensions with a large air cell. Egg shape is also highly variable which means that the material being measured would not have a constant distribution with respect to the field of applied energy. This variation would affect the amount of energy absorbed and thus affect the machine readings. Therefore, with respect to any one egg, its total conductivity effect would depend on the size of the egg, the size of the air cell, and the shape of the egg, as well as the conductivity of the contents.

Evaluation of Two R-F Egg-Testing Devices

The 2 devices tested were the "Electronic Candler" designed by Romanoff *(4)* and the "Electronic Egg-Testing Machine" by Gascoigne *(3)*. These units will be referred to as machine "A" for the Romanoff unit and machine "B" for the Gascoigne. Machine "A", operating at 67 mc., made a measurement of the current flowing in a coil surrounding the egg. Quality ratings were made by comparing this current with the current flowing when a standard was placed in the coil. The standard was an artificial egg, made of plastic, filled with egg albumen and sealed. It was intended to represent a high-quality egg that would not deteriorate with time. Machine "B", operating at about 70 kc., made a measurement of the voltage developed across a pair of condenser plates cupped about the ends of the egg. Here again quality ratings were made by comparing the voltage readings with the reading for a standard, but in this case a high-quality egg was used as the standard. In actual operation the machine was standardized by choosing the egg that gave the highest meter reading and using it as the standard.

Both devices were tested for accuracy of quality determination on day-old eggs. Repeated measurements were also taken on a group of eggs as they were allowed to deteriorate in quality under conditions which controlled air-cell development. Measurements were made of the machine reading, the size and shape of each egg, and the albumen height.

Figure 6 is a scatter diagram of machine readings plotted against albumen height for machine "A". Eggs with greatest albumen heights are, of course, of highest quality, and on this machine high readings are supposed to indicate low quality. The graph shows clearly that there is practically no relationship between albumen quality and machine readings. Figure 7 is a similar scatter diagram for machine "B", with the same con-

clusion being apparent. On machine "B" high readings are supposed to indicate high quality.

Figure 8 shows the relationship of average change in machine reading to shape index (width × 100 divided by length) for various weight classes of day-old eggs. These curves show that the machine readings varied approximately linearly with shape index with long narrow eggs giving the greatest change from standard on both devices. On machine "A", egg weight also affected the reading with heavier eggs giving greater change from the standard than lighter eggs. Machine "B" did not show the same sensitivity to egg size, and the average curve for all weights is the only one shown. As pointed out previously, results of this nature were to be expected since total conductivity effect is a function of volume (egg size) and distribution of the test material (egg shape).

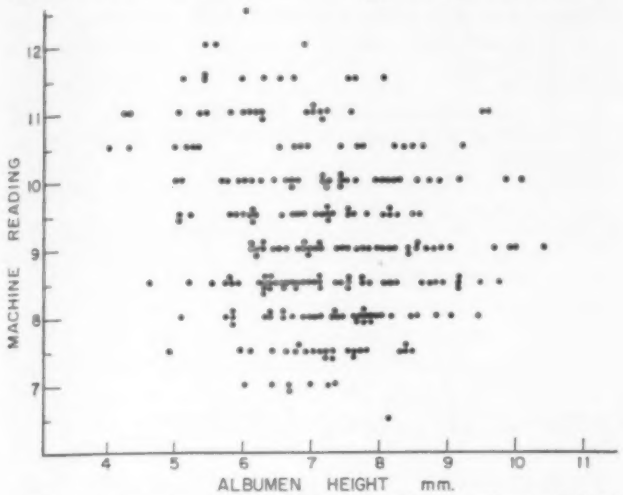

Figure 6. Relationship of machine reading to albumen height for day-old eggs, using machine "A".

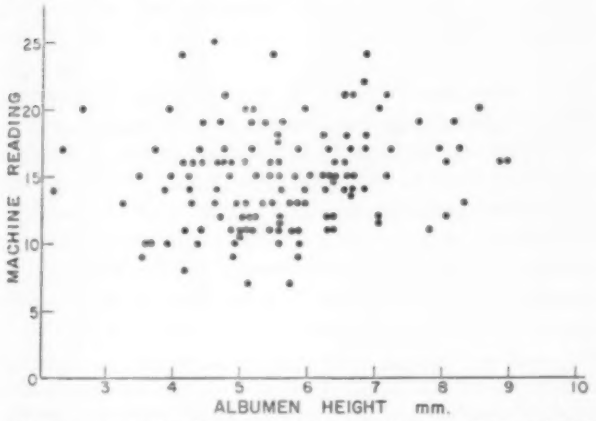

Figure 7. Relationship of machine reading to albumen height for day-old eggs, using machine "B".

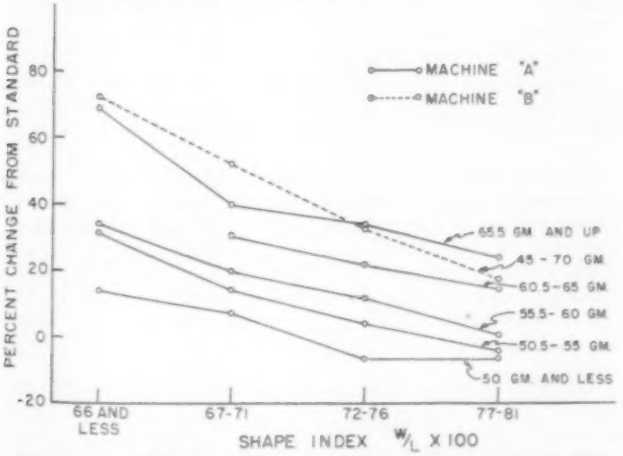

Figure 8. Relationship of change in Electronic Candler reading to shape index of various weight classes of day-old eggs.

To illustrate the effects of increasing air-cell, machine readings were taken on a group of day-old eggs. Some of the eggs were allowed to age at low humidity (45-50% R.H.) at 72-75° F. (22-24° C.), for 2 weeks while others were aged at the same temperature in air near saturation to prevent increase in air-cell size. Machine readings were taken at intervals during the aging period.

Figure 9 shows that the eggs held at low humidity, where the air-cell size was permitted to increase, gave marked increase in meter readings with aging time.

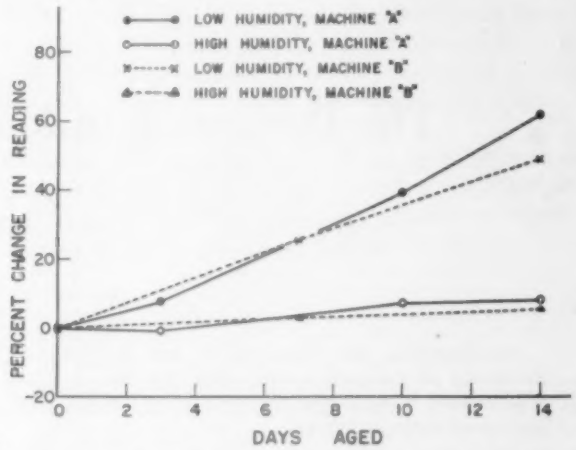

Figure 9. Relationship of change in machine reading to aging time for eggs held at high and low humidity.

Those aged under conditions where the air-cell size did not increase show little change in meter readings. Obviously, there were great losses of quality in all eggs. However, both machines indicated that only those with increased size of air cell were lowered in quality. These results are to be expected because of the change in volume that occurred under low-humidity aging as compared with high-humidity aging.

SUMMARY

Instrumentation is described for the measurement of the conductivity of highly conducting liquids over a frequency range of 50 kc. to 1,000 mc., using a coaxial cell, with the Boonton Q-Meter and the Kay Electric Company Mega-Match as the primary measuring elements.

With this instrumentation the conductivity of albumen from low-quality chicken eggs was found to be 8.5×10^{-3} mho per cm. at 72° F. (22° C.) for frequencies up to 50 mc. Above this frequency the conductivity increased with frequency. Yolk material from low-quality eggs was found to have a conductivity of 2.9×10^{-3} mho per cm. at 72° F. (22° C.) for frequencies up to 50 mc., with increasing conductivity above this frequency.

The conductivity of both yolk and albumen from high-quality eggs was found to be about 15% lower than the values for low-quality eggs. Each component was found to have a temperature sensitivity of about 1% conductivity increase for each degree Fahrenheit temperature increase.

Tests on the "Electronic Candler" designed by Romanoff and the "Electronic Egg-Testing Machine" designed by Gascoigne gave results in agreement with the conductivity measurements. Machine readings on individual eggs were found to be poor indications of interior quality, because of the effects of other variables such as egg shape and size and air-cell size. Each of these variables was found to produce changes in the machine readings as large or larger than the changes produced by quality differences. It is concluded that with the equipment now available, radio frequency conductivity measurements cannot be used to indicate correctly the interior quality of shell eggs.

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The Darkening of Maraschino and Glacé Cherries

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(Manuscript received January 14, 1952)

Investigations were conducted to find the cause of darkening of maraschino and glacé cherries. A practical method is suggested for the prevention and elimination of such darkening.

During the fall of 1950 glacé cherries which previously had a bright appetizing color turned a reddish black during the processing operation, and after packaging for a short period of time they turned almost black. Also the maraschino cherries darkened sufficiently to be unappetizing. This darkening effect applied to the entire cherry and not to isolated spots such as stem holes, blemishes, etc.

Work on this problem showed 3 major factors resulting in a discoloration of cherries. They were tannin complexes, heavy metals such as copper and iron, and oxygen from the air.

Tannins are a heterogeneous group of complex compounds of common occurrence in plants generally being of the catechol tannin group. Experiments conducted in the present case showed that the tannins present in the cherries under discussion, were also of the catechol type as indicated by the greenish-black coloration given with ferric chloride.

Since tannin structures contain many hydroxy groups, in the presence of oxygen and in an acid medium (pH of 3.0-4.0) one can readily see the tendency of heavy metals to react with the tannins and their derivatives. In the case where enzymes may break down the glucoside molecule, the resulting catechols and gallic acid will also react with these metals. Pyrogallol solutions with as little as 0.008% oxygen by volume turn a brownish black color *(6)*, and this blackness is further enhanced by presence of heavy metals.

Tannins are present in the cell sap, but occur most frequently in the cell walls, often accumulating in very considerable amounts in dead tissue *(4)*. Unripe drupe fruits such as the cherry, plum, almond, peach, etc. contain relatively large amounts of tannins *(3)*. As cherries for processing must be firm, they are often picked before reaching maturity.

Recent investigations in Italy showed that some cherries darkened when processed on account of the tannin originating from the chestnut wood both in the baskets when the cherries are picked and from their storage in chestnut barrels. For this reason, barrels made of oak and chestnut wood should never be used in storing cherries as the concentration of tannin is exceedingly high. Attempts have been made to "de-tanninize" the barrels using wax or a similar compound as a liner. The term de-tanninize, as commonly used by barrel manufacturers, does not actually indicate a removal of tannin from the barrels, but rather protects the cherries and the brine from contact with the wood.

Unfortunately, the barrel staves must be opened at one end to place the head into position, hence here the wax often becomes broken and the tannin at this area can contaminate the cherries. Also, pieces of wax have been known to break away from the staves through improper cleaning before waxing or through rough handling during transit. Beechwood barrels have been shown to have the smallest amount of tannin for an economical barrel.

During storage a properly coopered barrel will lose approximately 40 p.p.m. SO_2 per month, but a greater loss will occur when the barrel is unsound. If the SO_2 content drops too low (approximately 400 p.p.m.), the enzymes present may cause the catechols to be oxidized

which causes further darkening of the cherries. This effect is noticed by a grey appearance of the fruit instead of a straw color *(1)*.

Cocktail cherries, being processed with the stems, should be treated with great care as there is a considerable amount of tannin present in the stems.

Copper and Iron Are Chief Contaminants

Cohee and Nelson *(2)* have shown that iron was often present in the syrup line dippers used in handling the fruit, rust from barrel hoops, spray residues, calcium carbonate, brine, corn syrup, water supply, and the like. This work was investigated and verified by the present writer.

Copper contamination is also extremely bad for discoloration of cherries. The main source of copper is copper equipment. Experiments conducted in cooperation with the American Can Company at Hamilton showed that copper contaminations as low as 5 p.p.m. caused darkening effects both on the dye and cherry. At 10 p.p.m. of copper the cherries were quite dark, while at 25 p.p.m. the cherries were nearly black. Here too the concentrations of tannin played an important part in the intensity of the darkening effect. Relative concentrations of tannin were determined by intensity of color using dilute ferric chloride solutions. Copper and copper compounds will dissolve very readily in glucose solution more so than other metals *(5)*.

Another important factor to consider is that copper promotes the oxidation of sulphurous acid to sulphuric acid. If sodium benzoate is used as a preservative, it is necessary to add citric acid to give a pH of 3.0-3.5 for activation of the benzoate. This acid condition is ideal for dissolving oxides of copper from the kettles or other equipment. Here too concentrations of copper can be built up by continually reusing syrups for glacé cherries.

Atkinson and Strachan *(1)* have made exhaustive tests of the effect of using kettles and equipment of various materials for processing cherries. The following were graded from the best type to the least desirable: glass, stainless steel, nickel, aluminum, tin, monel, and copper.

As little as 7 p.p.m. of copper will cause pure food dyes such as Ponceau 3R and SX, Amaranth, Erythrosine, light Green S. F. Yellowish, etc. to darken. Primary green colors have a tendency to turn a brownish green if copper contamination is over 8 p.p.m. Adding more or less dye does not materially enhance the color of the cherries in a darkened state.

Stainless steel kettles are gradually replacing copper kettles. However, the heat transfer with copper is 1.77 times as fast as with stainless steel, based on same thickness and steam pressure. Stainless steel kettles for syrups using sodium benzoate usually contain 18% chromium, 8% nickel, and 0.08% carbon. Where large quantities of SO_2 are used, the addition of 0.25% molybdenum increases the resistance to wear.

Elimination of tannins, iron and copper plus oxygen will aid in prevention of the cherries from going dark.

Removal of Contaminants

In 1951 Cohee and Nelson *(2)* showed how iron could be removed by heating cherries in a 50% syrup with 0.2% calcium phytate at a pH of 3.0. A white precipitate of iron phytate forms which can be filtered off. At a pH of 3.0, calcium phytate is in equilibrium, with phytic acid which reacts with the iron (or copper) present.

The removal of iron and copper was tried by the author and proven successful if there were no large amounts of tannins present. The Canadian Food and Drug Act at first did not permit calcium phytate to be used in the processing of maraschino or glacé cherries. Possibly this was due to calcium phytate being an ester of inositol which is found in plant and animal tissue and is a vitamin of a type. Regulations now permit its use providing there is no calcium phytate left in the finished product.

Another approach to the problem of restoring the bright color to the cherries is to add 15 pounds of sulphurous acid (6% SO_2 dissolved in water) before the last syruping whether maraschino or glacé cherries, to 100 pounds cherries net weight. The cherries, syrup, acid, etc. are boiled in stainless steel kettles to desired temperature. This final boiling should remove all of the SO_2 in the cherry and syrup. Rapid cooling of cherries and syrup helps to retain the shape and prevents shrivelling.

Sulphur dioxide being a reducing agent, it reduces the tannin or the metal-tannin complex already formed, and as long as oxygen can be prevented from re-entering the cherry to cause oxidation, the cherry will remain of bright color.

Here then is another problem unsolved. No successful antioxidant has been placed on the market to retard the oxidation of tannin complexes in cherries. Sulphur dioxide is used by many packers, but this gas gradually disappears, being replaced with oxygen, in particular with glacé cherries. The author has also used the above SO_2 treatment as described above on preserved lemon and orange peel, and has found the color greatly enhanced after the treatment.

SUMMARY AND CONCLUSION

Darkening of maraschino and glacé cherries can be prevented by avoiding the contamination of cherries with tannin from various woods, and of contamination by iron and copper from the equipment used in the processing operations.

In the case where the cherries have already darkened after processing, iron and copper can be removed by using the calcium phytate treatment, and dark-colored tannin complexes can be reduced by using the sulphur dioxide treatment.

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Recovery of Fruit Essences in Preserve Manufacture

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(Manuscript received February 1, 1952)

This paper presents a method for recovering, in essence form, the fruit aromas normally lost in the conventional vacuum-pan processes of making jams and preserves. A surface condenser is used instead of the usual water-jet or barometric condenser, and its condensate is processed to form a strong essence. The essences thus obtained from strawberry, cherry, peach, and blackberry have the characteristic odor of the cooking fruit. They can be put back into the preserve to enhance its flavor, or used as flavoring materials for other fruit products.

In the usual procedures for making fruit jams and preserves, which involve boiling off 10 to 30% of the water contained in the fruit, most of the substances which give fragrance to the fruit are driven off with the steam. Our grandmothers' kitchens bore odorous testimony to the loss incurred when the fruit was boiled in open kettles, and in vacuum pans the only difference is that the fragrant substances are lost in the condenser water instead of to the atmosphere. The substitution of a surface condenser for the more usual water-jet or barometric type, and the use of low-temperature water for the condenser or for an after-cooler, constitute the first step in recovering this loss. The next step is concentrating the collected condensate in an essence-recovery apparatus based on the principles developed at the Eastern Regional Research Laboratory for recovering and concentrating essence from fruit juices. By this procedure nearly all the aroma otherwise lost from each hundred pounds of preserves can be concentrated into less than a pint of essence. It is to be noted that the manufacture and use of such essences ("volatile flavor concentrates") are subject to regulations of the Bureau of Internal Revenue *(1)*.

Reincorporation of essence into preserves[b] improves their flavor and aroma, giving the preserves a stronger and more fruity character. Tests now under way on storage of these preserves at room temperature indicate that, at least with certain fruits, the improved flavor does not last many months. A manufacturer whose normal processing gives products of adequate quality for his market might profitably sell the essences as by-products. There is now a market for volatile fruit concentrates for contributing true fruit flavor in the manufacture of beverages, fountain sirups, ice creams, and confectionery.

In pilot-plant work at the Eastern Regional Research Laboratory, application of this process to cherry, strawberry, peach, and blackberry preserves has been investigated. In each case, an essence of good aroma was obtained. Strawberry, blackberry, and cherry were strong and excellent. The peach preserve was made, as usual, from canned peaches; hence the essence had that characteristic odor and was not very strong.

[a] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

[b] The restoration of aroma to preserves by the reincorporation of essence should be cleared with the Food and Drug Administration for compliance with the standards of identity for these products.

EXPERIMENTAL

Condensing the vapors. In making a typical 2000-pound batch of preserves, the water evaporated may be about 380 pounds. For instance, the manufacturer may mix in an open steam-jacketed kettle 1240 pounds of frozen sugared cherries previously thawed (containing 1 pound of sugar per 4 pounds of fresh cherries), add 932 pounds of sugar, 12 pounds of dry pectin dissolved in 181 pounds of water, and 15 pounds of 50% citric acid solution, heat the mixture to 150° F. (65.5° C.) and stir for 5 minutes. The hot mixture is drawn into a vacuum pan, and the vacuum is raised to 25-28 inches; some evaporation thus occurs spontaneously. Steam is then turned into the jacket, and evaporation is continued until the density of the sirup reaches about 63° Brix. The vacuum is then broken by letting air into the pan; this is to force the sirup into the interior of the individual cherries. Next the vacuum is restored, and evaporation continued to about 67° Brix. Once more the vacuum is broken and restored, and when the density reaches 68° Brix the batch is discharged to an open kettle, where it is heated to 180° F. (82° C.) for packaging. Steam is kept in the jacket during this entire period of cooking in the vacuum pan, which usually takes 45 minutes. Carry-over or entrainment of liquid with the vapors should, of course, be avoided as far as possible, for this might cause fermentation of the condensed vapors unless they are processed the same day.

The water vapor evolved from the batch (in the quoted example amounting to 380 pounds) carries away with it most of the odorous substances originally present in the fresh fruit or developed during the cooking. To recover these substances, the present process uses a tubular surface condenser to condense the vapor, instead of the customary type of condenser in which the vapors are directly mixed with water and thus discarded. Since many of the odorous substances have low boiling points, the condenser must be so designed as to cool the condensed vapor as low as feasible and thus minimize the loss of these volatile substances. The frequent breaking of vacuum introduces a considerable quantity of air into the vapor going to the condenser, and of course this air as it leaves the condenser carries some volatiles away with it. Since the air leaves saturated with respect to the liquid components of the condensate, it must be cooled in order to reduce the loss of volatiles to a small amount. This also calls for consideration in designing or choosing the condenser. It should be of the type in which the vapor is inside vertical tubes, and the shell should be well baffled so as to give the cooling water a definite path countercurrent to the flow of the vapor. Thus both the condensate and the air in the latter part of their travel can be well cooled by the incoming water. The vertical type gives the best cooling of the condensate, since it flows down over the entire surface of the tubes instead of along the bottom as in horizontal or inclined tubes. The type of condenser having the vapor outside the tubes is undesirable because of the impossibility of properly cleaning the vapor and condensate spaces. As discussed later, the degree of cooling necessary for satisfactorily complete recovery is different with different fruits; it is in the range of 60-80° F. (15.5-26.5° C.). The degree desirable in any particular installation is an economic question, varying with the availability and cost of low-temperature water.

If a manufacturer happens to have a small supply of very cold water in addition to a large supply of somewhat warmer water, an attractive arrangement is the use of a separate after-cooler, through which condensate and air pass on leaving the main condenser, the after-cooler being supplied with the coldest water and the water leaving it being added to the supply of water for the main condenser. The after-cooler should have vapor passages of liberal cross-sectional area to avoid high frictional resistance when the vacuum is being raised. If multitubular, it should be arranged so that the incoming condensate is well distributed among the various tubes.

The apparatus used in our experimental work (Figure 1) included more items of equipment than would be used in a commercial plant. To determine how much cooling of the condensate would be economically desirable, we used an after-cooler. Further, to determine the amount of aroma escaping from the after-cooler in the vent gases, we passed them up through a scrubbing tower. Cold water flowing down through this tower absorbed the residual aromatic constituents for later evaluation. In making blackberry preserve, cooling the condensate only to 82° F. (28° C.) was adequate to reduce to a commercially negligible amount the loss of volatiles in vent gas from the after-cooler and obtain an essence of good character. With strawberries, however, it was necessary to cool the condensate to about 62° F. (16.5° C.) to achieve this result. With peaches (canned as is customary for preserve manufacture) and with cherries, cooling to 62° F. was satisfactory, but the necessity of cooling this low was not established. These temperature limits were arrived at by odor and taste tests, made by a trained panel in a manner similar to the tests for vaporization described later.

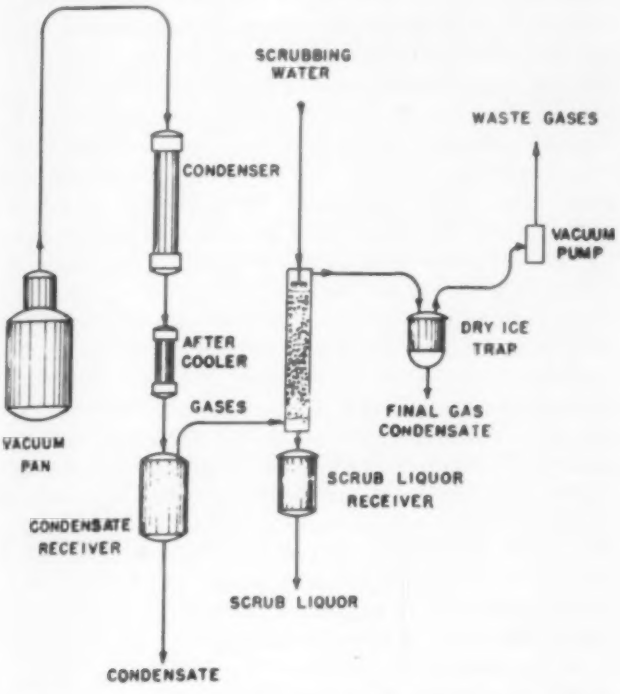

Figure 1. Apparatus for investigating recovery of aroma lost in the manufacture of preserves.

To achieve cooling to 62° F. requires water at 50-55° F. (10-13° C.) in sufficient quantity to operate at least an after-cooler. Many manufacturers may have such a supply in winter, and hence could obtain a high yield of satisfactory essence from the four kinds of fruits we have tested, and probably from other fruits not yet investigated, without resort to the other devices described below. If such cold water is not available in summer, as from deep wells or by refrigeration, high essence recovery from some fruits, such as strawberries, could not be obtained by this method, but could be had by vent gas scrubbing, as explained later. Refrigeration of the small quantity of water needed for an after-cooler is not impracticable. For instance, if the temperature of the main water supply is 75° F. (24° C.), each ton of preserves would require only about 60 gallons of water at 50° F. (10° C.) and production of 1 ton per 45 minutes would require a 2-ton refrigerating plant, with a 2-horsepower motor.

An alternative scheme for operating without low-temperature water is the use of a scrubbing tower for the air and gases vented from the condenser; in this case no after-cooler is needed. The vent gases, carrying, of course, a certain amount of vapors, pass up through the tower while fresh water at normal temperature flows down. The water leaving the bottom of the tower is collected and added to the condensate for processing into essence. If all the air exhausted from the vacuum pan during the raising of vacuum were passed through the scrubbing tower, a tower of an unnecessarily large diameter would be needed, which would require an excessive amount of water. Therefore, since this air contains very little aroma (because the contents of the pan are not boiling) the tower can be bypassed and the air sent directly from the condenser to the vacuum pump during these periods.

Making the essence. In order that the aroma have commercial utility as a source of flavor, it must be concentrated to essence. This can be accomplished by processing the condensate, or condensate plus scrub liquor, by the method developed at this Laboratory for recovery of essence from fruit juice.

Apparatus to produce essence directly from fruit juices has been described in previous publications of the Eastern Regional Research Laboratory *(6, 2, 3, 4, 7*[e]*)*. For recovering essence from condensate, the process will be somewhat simpler. Since the condensates are not as sensitive to heat-damage as are fruit juices, the preheater and vaporizer need not be designed for such high velocities as fruit juices require. The stripped condensate from the separator can be discarded and therefore need not be cooled. The vent gas need not be scrubbed with water (obtained from the bottom of the fractionating column). It is sufficient to chill thoroughly the vent gas, primarily to assure condensation of its accompanying volatiles. The chilling can be accomplished most simply by passing the gas up through a small packed tube down which flows chilled essence. This tube and a small precooler for the essence are immersed in an ice bath. This simplified essence recovery system is shown in Figure 2. The essence apparatus works continuously, not batchwise. The general principle is as follows: the feed liquid (condensate containing the aroma) is pumped at a constant rate through a preheater, which heats it to its boiling point, and through a vaporizer in which a chosen percentage of it is vaporized, each controlled by adjusting its steam supply. The mixture of vapor and residual liquid is sent to a separator, from which the liquid is discarded by means of a tight-closing steam-trap and the vapor sent to a fractionating column. By means of the column with its condenser and reflux splitter, most of the water is discarded as column bottoms, and a concentrated essence is produced. The air that enters with the feed liquid is vented from the reflux splitter and chilled before being discarded to the atmosphere.

Determination of the proper percentages to vaporize out of the various condensates was made by odor tests of the essences and the liquids from which they were stripped. The percentage chosen did not necessarily represent complete recovery of all volatile constituents in the distillate, for complete recovery would be uneconomical in commercial practice. Recovery was judged adequate when the essence had the character of the fruit from which it was derived and an essence made from a second stripping of the stripped condensate was of only very low potency. For cherries and peaches, the vaporization thus arrived at was 20%, for blackberries, 30%, and for strawberries, 40%. For any one fruit, these figures may conceivably vary according to the variety of the fruit; the varieties we used are discussed later.

[e] In the article cited under 7 there is an important error. On page 2, column 2, line 12, it should read "0.104-inch" and not "0.014-inch."

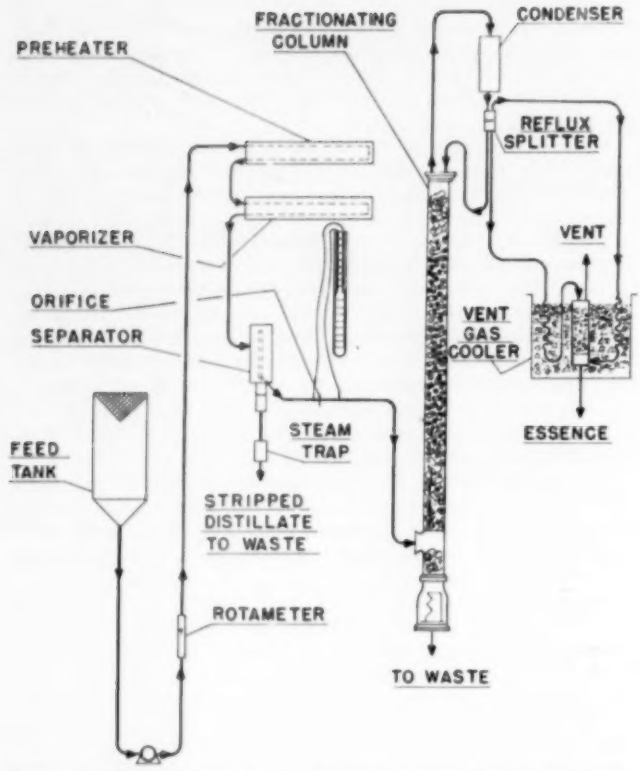

Figure 2. Apparatus for producing essence from distillates.

The strength of the essence produced can be adjusted by merely changing the volumetric rate at which it is drawn off from the reflux splitter; doubling this rate will produce twice as much essence, but of only half the strength. For instance, in the example given, the 380 pounds of condensate can be made to yield 9.5 pounds of essence, which will therefore be 40 times as strong in aroma as the condensate, or nearly so. If the drawoff be adjusted to produce 19 pounds of essence from 380 pounds of condensate, the essence will be only 20 times as strong as the condensate. A convenient commercial figure for expressing the strength of the essence is the number of pounds of preserve from which 1 fluid ounce of essence is made. This figure can be called "potency"; in the example given, recovering 9.5 pounds of essence (that is, 146 fluid ounces) from 2000 pounds of preserve, the potency of the essence would be 2000/146, or 13.7. In previous publications dealing with the production of essence from fruit juices, the term "fold" has been used to designate the strength of the essence. For instance, "150-fold essence" means that 1 gallon of essence contains all, or nearly all, the aroma from 150 gallons of juice. This concept cannot be applied to essences derived from preserves, as it implies substantially complete recovery of aroma from the juice or fruit. Obviously, some aroma remains in the preserve. This work is directed only to recovering that which is normally lost.

Storage and utilization of the essence. If the essence is to be reincorporated into the preserve, it is of course not essential to good flavor that each lot of essence be used with the kettle-batch of preserve from which it was obtained. Since the essence-making apparatus is inherently continuous-acting, and takes 15 to 30 minutes to come to equilibrium, the best scheduling is to run it a whole day on condensate from one fruit rather than to switch frequently from one fruit to another. If it becomes necessary to hold condensate overnight it should be kept under refrigeration to prevent the possibility of fermentation. The essences will normally be free from sugar. Nevertheless it is recommended that essences be kept in cold storage, in well-filled and tightly stoppered containers, to minimize the possibility of deterioration.

RESULTS OBTAINED ON VARIOUS FRUITS

Cherries. Montmorency cherries, packed frozen with 1 pound of sugar per 5 pounds of cherries, were used. The batch was concentrated at 25 inches of vacuum from 61° Brix to 71° Brix, the vacuum being broken and restored twice during the concentrating. The batch was then heated to 180° F. (82° C.) and drawn off into glass jars. The condensate was cooled to 64° F. (18° C.) by the condenser, hence no separate aftercooler was used. The condensate, which amounted to about 18 pounds, had a good strong odor typical of cherry preserve. The vent gas from the condenser was scrubbed with chilled water totaling 11 pounds; the resulting scrub liquid had so little odor that even a 25-fold essence made from it had a negligible amount of aroma. Hence the conclusion was drawn that if the condensate is cooled to 62° F. scrubbing of the vent gas is unnecessary. For experimental confirmation, the vent gas was sent from the scrubbing tower to a dry-ice trap; the liquid collected here was odorless and a chemical test (titration with alkaline permanganate solution) showed absence of organic substances, proving the adequacy of the scrubber as an indicator of loss, if any, from the condenser.

The condensate was processed in the essence-recovery apparatus, vaporizing 20% and making an essence of 25-fold with respect to the condensate. This was found to have in a very strong degree the odor characteristic of cherry preserves.

The stripped condensate, that is, the 80% remaining after passing through the vaporizer of the essence unit, had a definite cherry aroma. It was, therefore, processed to make a 25-fold essence, but the essence produced had so little aroma as to be considered not worth the expense of recovering it in commercial practice. In spite of its little odor, however, analysis by alkaline permanganate titration showed that it contained 23% of the total organic material originally present in the condensate. This illustrates the fact that chemical determination of total organic substances, as for instance by permanganate titration, although useful for certain experimental purposes, does not give a true indication of the relative value of the actual aroma of different products. For instance, ethyl alcohol, a preponderant component of most essences, responds to the permanganate test but actually contributes little or nothing to the aroma.

Addition of the essence from the condensate to the preserve made in the vacuum pan produced marked improvement in both aroma and taste. The flavor was more fruity, having more of the character of the fresh cherry. The essence itself had a fine cherry odor. It

should prove a valuable and salable byproduct for a preserve manufacturer whose market does not demand, nor pay for, an improvement in quality over his standard preserve.

Peaches. For starting material, canned peaches were used, in accordance with customary practice, to avoid the darkening that fresh peaches would undergo in preserve manufacture. Using a mixture of 25% Elberta and 75% Clingstone peaches, we made a preserve and collected the distillate at 62° F. (16.5° C.). It had a good odor of canned peaches. The intensity of the odor was of course much less than that of cherry essence, since canned peaches themselves have little odor. An essence made from the effluent liquid from the vent gas scrubber contained little aroma; therefore, it was not considered commercially worth while to scrub this gas. Vaporizations of 20, 25, and 30% were given to the distillate in the essence apparatus. The stripped distillates were all very weak in odor and practically indistinguishable from each other, showing that no increase in recovery of aroma would be obtained by vaporizing more than 20%. A batch of essence made from the distillate at 20% vaporization was returned to the preserve from which it had been obtained. This gave a definite improvement in both aroma and flavor.

Strawberries. With strawberry preserve, trials were made to determine whether distillate temperature as high at 76° F. (24.5° C.) would give adequate recovery without scrubbing. A frozen sugared-pack of Marshall berries was used (3 parts of berries to 1 of sugar). When cooling the distillate only to 76° F., the liquid from the vent gas scrubber had a fairly strong odor. An essence made from this scrub liquid (using 20% vaporization) had a strong odor of strawberry jam. Therefore, if the distillate is collected at as high a temperature as 76° F., scrubbing the vent gas is necessary for good recovery of aroma. A vaporization of 40% in the essence apparatus is adequate to recover substantially all the aroma present in the combined distillate and scrub liquor.

Cooling the distillate and vent gas to 62° F. (16.5° C.) gave satisfactory recovery of aroma without scrubbing the vent gas. Also 40% vaporization was necessary in stripping the distillate in the essence apparatus; the gain by using 50% was insignificant. An essence so made, when returned to the preserve from which it had been obtained, enhanced the flavor and aroma to a marked degree; the resulting full-flavor preserve had an aroma and flavor stronger and more characteristic of fresh strawberries.

Blackberries. Low cooling of the distillate and vent gas was not necessary with blackberries; a temperature of 82° F. (28° C.) was adequate to reduce vent gas loss to a very small amount. The distillate had a fine strong odor similar to that of the preserve. A vaporization of 30% in the essence unit was required, and a 25-fold essence thus made appreciably improved the preserve in aroma and flavor. An interesting point here was that the full-strength essence had an odor somewhat different from that of the preserve, but when diluted with water it regained the true odor of preserve. It is not always possible to judge the value of an essence by smelling it at full strength. Blackberry essence recovered as described above can be a potent source of blackberry flavor.

CONCLUSION

By the methods and essence-recovery apparatus presented in this paper, a manufacturer of fruit preserves and jams can recover, in the form of concentrated essence, fruit aromas which are lost in the conventional method of manufacture. The essences could be sold to manufacturers of flavoring materials or other fruit products, or can be put back into the preserves to enhance their flavor.

In winter, when at least a part of the water supply is at 50-55° F. (10-13° C.), excellent recovery of aroma from the vapors can be obtained without refrigeration or scrubbing, hence with the minimum investment for equipment. In summer, with water temperatures of 65-75° F. (18.5-24° C.) some fruits (blackberries) will give excellent recoveries. With other fruits (strawberries), the recovery of aroma is less complete unless the condensed vapors are cooled to 60-65° F. (15.5-18.5° C.) or the vent gases from the condenser are scrubbed. The cooling will need a small supply of water at 50-55° F.; scrubbing will require the installation of a small packed tower and a supply of odorless water at normal temperature.

The experimental work thus far done on 4 fruits (strawberries, cherries, peaches and blackberries) has indicated the desirability of further trials on a larger scale, especially with additional fruits. Such trials, utilizing vapors evolved from vacuum pans in an actual preserve manufacturing plant, are planned as part of the continuing research program of the Eastern Regional Research Laboratory. They are expected also to provide a sufficient quantity of essences for commercial evaluation in food products other than preserves.

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Relative Color Evaluation of Food Purees Using Black and White Photography

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An objective method for the color comparison of food purees by means of black and white photography is described. The method can be extended to non-uniformly colored samples.

One of the very obvious advantages of glass as a container material for processed foods is its transparency. The "eye appeal" of the product within is generally acknowledged to be an important factor in influencing the buying habits of the general public . This advantage can become a disadvantage if the enclosed product undergoes discoloration.

A special type of discoloration is that termed "graded discoloration." This type of discoloration, which causes the product to have a stratified appearance, has been especially costly to the baby food industry and in 1950 leading domestic manufacturers of baby foods launched a comprehensive cooperative research program directed at a practical solution of the discoloration problem.

These laboratories were assigned that phase of the whole problem which has to do with investigating discoloration of food purees in glass tubes sealed under carefully controlled conditions. It soon became evident that success in these investigations would be directly contingent on the development of objective methods for evaluation of the discoloration.

Discoloration of a food puree in a glass container is a time function and is known to take place over periods ranging into months and perhaps years. The human eye is quite accurate when the problem is one of simultaneous color comparison. This is not the case when delayed comparisons are attempted. Accurate direct comparison of colors in a jar of peaches today with similar colors in a jar seen 3 months previously is quite difficult.

Permanent intermediate standards such as the Munsell System, Ostwald Color Chips, Maerz and Paul Color Dictionary, etc., would be of assistance but, even then, color estimation would be quite subjective and vary with the observer. The fact that color varies with depth in the container as in graded discoloration is still another complicating factor. Objective instruments based on photo-tube circuits and the tristimulus-standard observer theory are available and might possibly be adapted for use. Investigation has shown that very extensive modifications in both instruments and associated techniques would be necessary before they could be used in the non-destructive evaluation of graded or zoned discoloration of food purees in glass containers.

A rapid, non-destructive, objective, method for the color comparison of food purees in glass has been therefore developed and investigated and will be described in this paper.

EXPERIMENTAL

Apparatus

A 4 × 5 Speed Graphic equipped with a 135 mm. Graflex Optar lens was used. Large negatives with a minimum of distortion were produced through the use of this camera-lens combination.

A simple 2-tube holder was constructed. The photography of more than 2 tubes at small objective-lens distance is not recommended because of inherent illumination problems. The holder was surfaced to a dull black in order to minimize stray reflections resulting in unwanted highlights on the negatives. Sample tubes and holder are shown on the left side in Figure 1.

Figure 1. Equipment used in black and white photographic color evaluation method. Tube holder on left. Densitometer-mechanical stage assembly on right.

Sufficient illumination was furnished by 2 RFL No. 2 photoflood lamps positioned about 30 inches behind, 30 inches apart, and 30 inches above the tube holder.

Although rated by the manufacturer as having a 6 hour life, bulbs were discarded after only 2 hours of use in order to obtain maximum constancy in the spectral quality of the illumination. As will be demonstrated in a later section, change in quantity of illumination has little or no effect on the final results.

A positive, calibrated gray scale was purchased from the Eastman Kodak Co. Such a scale consists of a sheet (8 × 10 inches) of glossy photographic paper which has been divided into 10 or 15 step sections of varying shades of gray ranging from fully-exposed black to unexposed white. Calibration of the scale involves measurement of the light reflecting ability of each step and was done at the Eastman Company Laboratories.

A miniature scale, suitable for mounting on the sample tube holder, was constructed from the larger one and permanently mounted as shown in Figure 1.

Film of the same emulsion lot number sufficient for the total eventual project needs was purchased. "Ansco Isopan" was selected for use.

Color filters were used for purposes of increasing discoloration contrast. A Corning 4303 filter with maximum transmission at 470 mμ was used for apricots and peaches. Similarly a Lumetron M 490 with maximum transmission at 490 mμ and a Cenco 610 B with maximum transmission at 610 mμ were found useful with apples and beets respectively.

A simple blackened plywood frame for supporting lamp standards, camera, and tube holder was constructed for photography frame.

Eastman DK 50 and Eastman acid fixer were used for processing the film. The DK 50 was kept up to strength by periodic replenishment from unused stock. Film processing was conducted in a standard type of daylight developing tank.

A Weston photographic analyzer was used as a film density measuring device. Other instruments such as those made by Photovolt and Ansco would serve equally well. A Kogagu graduated mechanical stage was fitted with clips for holding film and was then mounted on the densitometer so that film could be accurately drawn past the densitometer scanning head. The sensitometric assembly with a typical negative in the mechanical stage is shown on the right side in Figure 1.

Methods of Measurement

The details of evaluating color in a sealed tube sample of Rome Beauty apple puree are given as an example in the following paragraphs.

The sample tube is mounted in the tube holder on the photography frame. A 3-minute exposure at *f*8 is made with a Lumetron M490 filter placed in front of the camera lens. The negative is processed in a 68° F. (20° C.) bath of DK 50 for 10 minutes with recommended agitation throughout. This treatment is followed by a water wash, a 15 minute acid fixer bath, and a 30 minute water rinse in the order given. After being thoroughly dried, the negative is mounted in the mechanical stage-densitometer assembly. Film density values as registered on the densitometer are recorded for the steps in the gray scale image and at measured intervals down the sample tube image. The gray scale values are taken twice, before and after the puree values, and are averaged for each step.

Calculations

Experimental values of the gray scale steps as reproduced in the negative are shown plotted against the previously calibrated reflectance (density) values of the same scale in Figure 2. The

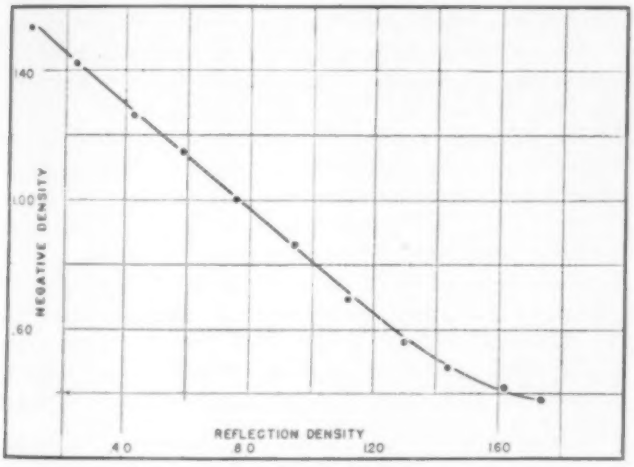

Figure 2. Negative calibration curve.

resulting curve mathematically compares the way a camera-film-densitometer combination "sees" a gray scale with the way a reflectometer "sees" the same scale.

Each negative is calibrated in this manner and density *(D)* measurement of other zones in the negative including, especially, the food puree image can be converted back into reflectance *(Dr)* values by use of the "calibration" curve.

RESULTS AND DISCUSSION

The photographic method rests on the simple premise that negatives of food purees will be blackened to a degree and in a manner depending on the color of the original puree. The photographic examination of several purees of variable color will result in negatives of variable density. If photographic conditions including film type, exposure, and development have been properly selected, the degree of blackness will be directly related to the degree of color. Small differences in color may produce very small differences in negative density; for this reason it is usually necessary to increase or amplify the photographic effect. This may be done by a, selection of film which is sensitive to the specific color change system of the puree under investigation; b, illumination during exposure by suitably selected colored light; c, use of high contrast film developers and darkroom techniques; and d, by use of high contrast film. Complicating factors are present in some of these alternatives while others may be difficult to realize experimentally. The use of alternatives b and c proved to be adequate in increasing color contrast as registered on film.

One of the major difficulties with the use of photographic methods in the past has been the lack of reproducibility of the results. Among the factors which cause the occurrence of non-systematic errors are variability in lighting and variability in film. In spite of efforts of film manufacturers to the contrary, film is usually quite variable in quality. Errors can be decreased through the purchase of film from one lot number, but the writer has found film to be variable even within a package. Variability in processing techniques is another disturbing factor and the extensive literature which is available on this subject serves to demonstrate the difficult nature of the task of obtaining conditions for absolute reproducibility in development. It has been found possible to very markedly reduce errors, ascribed in the foregoing sources, by an absolute comparison technique.

Reflection density Dr may be defined as:

$$Dr = \log 1/R \qquad (1)$$

Where 1 is the intensity of light reflected from the unexposed, processed paper base and R is the intensity of light reflected from any step in the scale while negative density, D, of a film may be defined as:

$$D = \log I_o/I_x \qquad (2)$$

Where I_o is the total incident light intensity and I_x is that transmitted through the negative. The non-ideal behavior of commercial photographic emulsions results in curves, the shapes of which are determined by film type, length of exposure, and film processing methods.

As noted previously, the puree sample and the gray scale are photographed simultaneously and on the same plate. Uncontrolled photographic variables will cause changes in general density and contrast range in the negative, but the method described in this paper rests on the assumption that these variables will not affect the proportionality relationships between the density of steps in the gray scale and that in the puree.

This treatment of the experimental data is completed by conversion of the puree negative density values into reflection density values by use of the calibration curve. Experiments in support of the foregoing will be presented in a following section.

In use, the negative density values for the puree sample are found on the D axis of the calibration curve.

The point of intersection of each value with the curve is read on the *Dr* axis. The data have, in this manner, been converted to a non-variable scale and are expressed in reflection density units.

Reflection density as a function of distance from the meniscus in both a discolored tube and a normal tube is shown in Figure 3. Discoloration can take place as

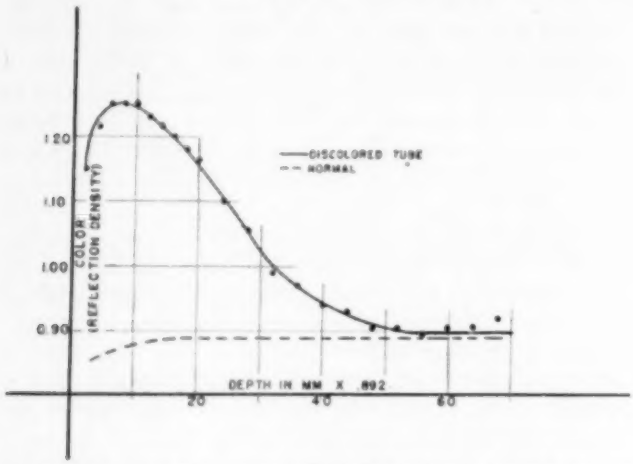

Figure 3. Color in apple puree-filled tubes as a function of puree depth.

the result of 1, the formation of color bodies from previously uncolored material; 2, the change in hue of already occurring pigments; and 3, the destruction to colorless bodies of previously occurring pigments.

Graded discoloration, a special case of discoloration, has a number of different attributes, most of which can be measured by the photographic method. The gradient, or sharpness of color transition within the sample is related to the slope of the curves in Figure 3. The total amount of color in the discolored zone is another characteristic and can be measured by determining the difference in area between the "normal" and the "discolored" curves. The maximum difference in color within a container is still another measurable characteristic and is simply the difference between the high and low points in the curve in *Dr* units.

It will be noted as in Figure 3 that the *Dr* $\times$ depth plot of an "undiscolored" puree is non-linear at low puree depth values. This is due to liquid lens, differential scattering, and non-uniform lighting effects in the vicinity of the puree meniscus. Some of these can probably be eliminated through the use of tube holders of better design, judicious use of black masking tape, and more elaborate lighting. These refinements in technique were not considered to be necessary in the present investigations.

Figure 4 illustrates the type of information which can be gained through use of the photographic method. The curves show the change in color of a sealed tube of Rome Beauty apple puree as a function of time. Total change in color, color gradient, and maximum and minimum color difference for this series was determined and is given in Table 1.

The total change in color was determined by means of planimeter measurement of the area between the curves in terms of *Dr* — mm. units.

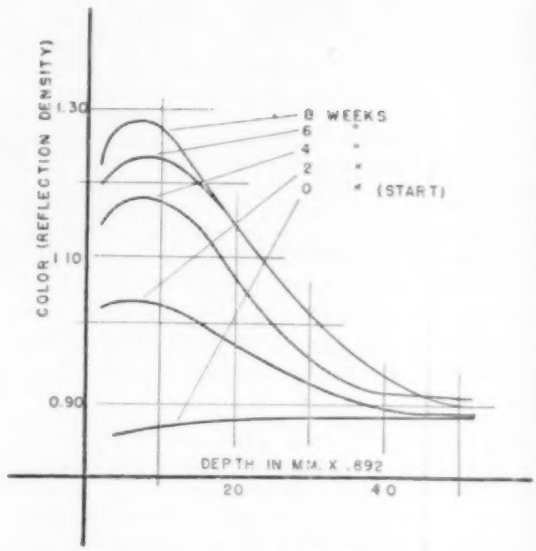

Figure 4. Color in apple puree-filled tubes as a function of puree depth and storage time.

"Discoloration gradient" was found by visually drawing the best straight line to the slope and then determining the tangent of the angle so created. "Discoloration range" is, as previously stated, the maximum and minimum in the discoloration curve.

It is to be pointed out that all of depth-in-mm. values in Figures 3 and 4 are those as found on the negative. The magnification factor of the camera-objective system as used was 0.892 by actual measurement, that is, values containing depth measurement should be multiplied by the factor 1/0.892 in order to get the value in terms of the real dimensions.

The methods of color comparison as developed in this paper will yield only relative results. Furthermore, their application is limited to color changes within a single system. The colorimetric behavior of pear and peach purees cannot be quantitatively compared. Color changes of a specific type, such as the darkening from yellow to red-brown of apples can be measured and can be considered to be a single color change system. The colorimetric effect of external influences on such a system can be measured. The reason for the limitation

TABLE 1

Changes in discoloration characteristics of apple puree as a function of time

Storage time in weeks	Total discoloration, *Dr* per mm.	Discoloration gradient, *Dr* per mm.	Discoloration range, *Dr units*
0	0	0	—0.03
2	3.71	—0.0057	0.15
4	7.40	—0.0112	0.27
6	9.56	—0.0123	0.33
8	10.2	—0.0125	0.39

is found in the fact that black-and-white panchromatic film is equally sensitive for all colors. The same negative density can be produced with various hues of widely varying nature. Black-and-white film is specifically insensitive to "hue" as defined in tristimulus colorimetry terminology and the film registers, in the main, the factors of chroma and value.

Errors

A statistical analysis of available data for errors and their magnitude was conducted. A small patch of gray paper was fixed to the tube holder and remained thereon for all photographs. With an ideal perfect system of evaluation the *Dr* values for this patch should be the same for every negative.

The *D* (negative density) and *Dr* (reflection density) value of this patch was determined on 48 negatives. Standard deviations were calculated for the *D* and *Dr* values and were found to be 0.035 and 0.017, respectively, where mean *D* was about 0.765 and mean *Dr* about 0.775. In terms of the three sigma confidence level it can be seen that the *Dr* values will be correct within $\pm$ 0.05 units in 19 out of 20 determinations.

In a further effort to determine the effect of unusually adverse conditions a series of pictures were taken of the same tube and gray scale. These data are summarized in Table 2 and show that variability in exposure and development times has substantially no effect on *Dr* values although under the same conditions large scale variation takes place in negative density and contrast factor (γ).

TABLE 2

Variability in lighting and development and its effect on photographic color evaluation results

Picture number	Change in development time [a]	Change in exposure time [a]	Gamma [b]	Average negative density [c]	Negative density of point A [d]	Reflection density of point A
	%	%				
103	0	+20	0.813	0.755	0.65	0.860
104	0	+10	0.796	0.723	0.62	0.875
105	0	0	0.839	0.705	0.59	0.885
106	0	−10	0.796	0.667	0.55	0.875
107	−20	0	0.735	0.680	0.58	0.880
108	−10	0	0.773	0.729	0.63	0.865
109	0	0	0.918	0.769	0.64	0.875
110	+10	0	0.980	0.815	0.68	0.863

[a] Exposure of 180 seconds at *f* 8; 10 minute, 68° F. development in DK50; were "100% is standard."
[b] Gamma is the slope of the "*D* Log *E*" curve and is the contrast characteristic of the film.
[c] Calculated from the gray scale.
[d] "Point A" is a point centered 20 mm. down from the puree meniscus.

SUMMARY

A non-destructive method of comparing color characteristics of food purees in glass by the use of black and white photography has been demonstrated. The use of color filters tends to make the method sensitive. The final conversion of data into reflection density units by means of a gray scale reduces the characteristic errors of a photographic method to a minimum. The method is particularly applicable to non-uniformly colored products and the measurement of graded discoloration in apple puree in glass is given as an example.

Acknowledgments

The assistance of Dr. H. P. Brady and the Stanford Research Institute in determining the feasibility and equipment requirements of the photographic color evaluation method is acknowledged. The author also wishes to thank various members of the Gerber Organization for constructive criticism and advice during the course of the investigations.

The Non-microbial Nature of the Black Spots on Ice-Packed Shrimp[a]

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(Manuscript received October 24, 1951)

Several different media, aerobic and anaerobic conditions, mass inoculations, and various chemical treatments of fresh shrimp were employed in an attempt to demonstrate a relationship between microorganisms and the development of black spots on shrimp. All efforts were unsuccessful and it was concluded that the blackening is not of microbial origin.

The development of black spots and blackened areas on fresh shrimp that are held in crushed ice has been a problem of the shrimp industry for many years. As described by Fieger, Colmer, and Alford *(2)* this discoloration usually begins within 2 to 4 days after storage as a blackening of the membrane which connects the ends of overlapping segments. One or more of these areas may blacken simultaneously, and if the shrimp is unheaded, the gills and walking legs usually blacken more rapidly than the segments of the tail section. By the fifth to eighth day the tail may have a definite banded appearance and disintegration of the chitin layer over the gills may have begun. The black spots usually develop more slowly and less extensively in headed shrimp, and not all shrimp in a catch will develop black spots. The appearance of typical black spots is shown in Figure 1.

Since these black spots are associated in the mind of the public with spoilage, shrimp showing this defect have a decreased market value. The present investigation was undertaken to study more fully the relation of microorganisms to this blackening as well as to investigate possible preventive measures. The occurrence of

[a] This research was supported by a grant from the Refrigeration Research Foundation, Colorado Springs, Colorado.

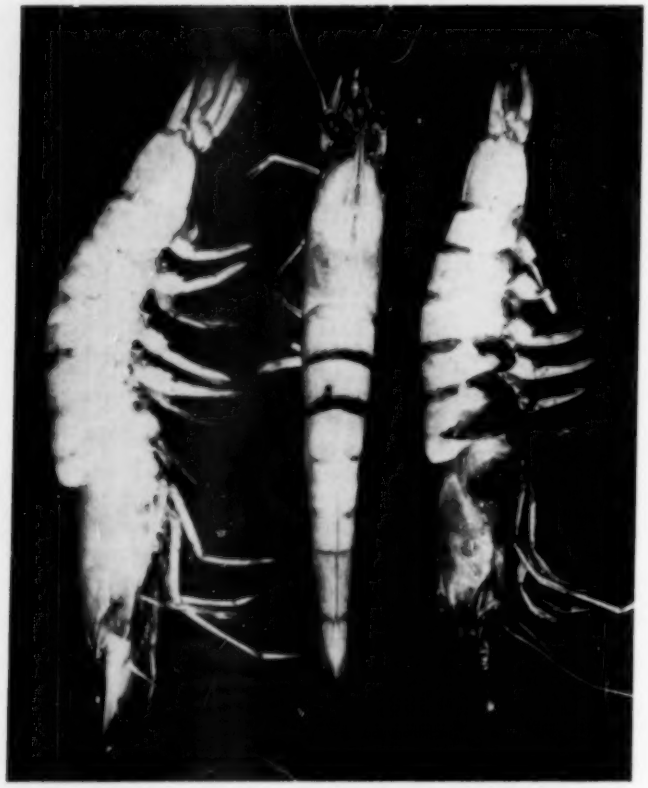

Figure 1. Appearance of black spots and blackened areas on ice-packed shrimp. All shrimp are from the same catch with identical conditions of holding.

black discolorations in meat and dairy products due to microbial action is well known, and some preliminary work *(1)* suggested that a mold might be involved as the cause of black spots in shrimp.

EXPERIMENTAL METHODS

The investigation of the importance of microorganisms in the development of black spots resolved itself into four phases: 1, isolation of microorganisms that might be the causative agents; 2, direct observations to determine whether any type of microorganism was more predominant in blackened than in other areas; 3, attempts to transmit the agent or agents causing spots to unspotted shrimp; and 4, attempts to determine factors that favor or inhibit the development of black spots.

Isolation of microorganisms. In an effort to isolate causative organisms of the black spots 18 different media were used. These ranged in complexity from a simple mineral salts-chitin medium to one containing tryptone, yeast extract, glucose, and shrimp peptone. The latter ingredient was prepared by the method of Snieszko, Griffin, and Friddle *(4)* for fish peptone. Sea water was used for the preparation of all of the media. Numerous blackened areas were removed aseptically from the shrimp, ground in a Waring blendor or with a mortar and pestle, diluted with sterile 0.85% saline and plated on the various media. Streak plates were prepared from similar samples. Replicate plates were incubated aerobically and anaerobically at 39° F. (4° C.), 68° F. (20° C.), and room temperature of 77-95° F. (25-35° C.) for 2 to 14 days. After suitable incubation, the plates were examined for black colonies or blackened areas on the medium. Representative colonies from many of the plates were isolated in pure culture for further study.

To investigate the possibility that the blackening might be a sulfide resulting from H_2S production by microorganisms, a quantitative estimate of the numbers of H_2S-producing bacteria in spotted and unspotted tissue was made in tubes of Difco Peptone Iron Agar. An additional check on the importance of H_2S production was made by placing unspotted shrimp in an atmosphere of pure H_2S for several hours.

Direct observation. Paraffin sections of spotted and normal tissues were prepared according to standard technics and examined to determine the relative numbers and types of microorganisms present.

Attempts to transmit black spots. If black spot development were the result of microbial activity, it should be possible to cause the blackening on normal shrimp by inoculation with spotted shrimp tissue or with the microorganisms suspected of being involved. Fresh, unspotted shrimp were washed several times with sterile water and then inoculated either with tissues containing black spots, the black "drip" from spotted shrimp, or with the bacteria or molds that had been isolated from media inoculated with blackened shrimp. Young cultures of the microorganisms were smeared on fresh shrimp in a similar manner. A Seitz-filtered extract of black spots was also used as an inoculum to explore the possibility of some filterable form of microorganism being involved. The inoculated shrimp were incubated in cracked ice at 32-34° F. (0-1° C.), at 39-41° F. (4-5° C.), or at 68° F. (20° C.).

Factors affecting black spot development. Headed and unheaded shrimp that were 24 to 72 hours old were treated with different concentrations of Roccal,[b] peracetic acid,[c] the sodium salt of dehydroacetic acid (DHA-S),[d] sodium proprionate, sodium bisulfite, propylene oxide, Krimko gel[e] plus ascorbic acid, or sterile water. The time of exposure and concentrations used are given in Table I. Some lots of the shrimp were washed twice with sterile water after exposure to the chemical, others were drained and stored without further treatment. Total bacterial counts were determined immediately after treatment on Zobell's Medium No. 2216 *(5)*. The treated shrimp were stored either directly in cracked ice (0-1° C.) or in cardboard boxes packed in cracked ice.

To determine the effect of oxygen on the development of spots, several fresh, unspotted shrimp were placed under anaerobic conditions in a Brewer jar and incubated in cracked ice. Control lots of shrimp were incubated similarly except that anaerobic conditions were not maintained in the Brewer jar.

RESULTS

From approximately 600 petri dishes of the various media that were inoculated with blackened and spotted pieces of shrimp, only one showed any blackening of the medium. In this instance a fungus was isolated. Neither this fungus, however, nor the mold that was isolated in the preliminary investigation grew rapidly enough at 32-39° F. (1-4° C.) to be considered as a causative agent of black spots. These fungi produced a blackening on heat-sterilized shrimp incubated at room temperature, but showed no blackening and little evidence of growth on fresh shrimp stored in cracked ice. By the use of chitin enrichments it was possible to isolate organisms capable of bringing about partial lysis of chitin strips within 7 days when incubated at 4° C. None of these organisms, however, produced blackening on any of the media. Johnson *(3)* has reported that certain chitinolytic myxobacteria isolated from crabs would cause a blackening on chitin media.

H_2S-producing bacteria were found in most of the samples examined, but the numbers present were not significantly greater in blackened areas than in unblackened areas. None of the fresh shrimp exposed to an atmosphere of H_2S showed any increased blackening.

[b] Furnished through courtesy of Sternwin Chemicals Co., New York, New York.

[c] Furnished through courtesy of Buffalo Electro-Chemical Company, Buffalo, N. Y.

[d] Furnished through courtesy of Dow Chemical Company, Midland, Michigan.

[e] Furnished through courtesy of Krimko Corporation, Chicago, Illinois.

Most of the organisms appearing on the plates that were incubated anaerobically were actually facultative in their oxygen requirements. The shrimp stored under anaerobic conditions in cracked ice did not develop black spots even after 10 days of storage, whereas the control shrimp developed them in the usual manner. As a result of this observation, the search for the causative organism among the anaerobes was discontinued.

Examination of paraffin sections of black spots failed to show any concentration of bacteria in the blackened areas, and there was no evidence of mold filaments in the tissues.

Although several hundred fresh shrimp were inoculated with blackened tissues or with microorganisms isolated from blackened tissues, there was no difference in the rate or extent of blackening on inoculated and uninoculated areas of the same shrimp or between inoculated shrimp and uninoculated ones. The black spots developed a little more rapidly on the shrimp held at 4°C. than on those held in cracked ice (0-1° C.). At temperatures of 20° C. and higher, however, no blackening developed. Bacterial growth on the shrimp held at the higher temperatures was quite rapid and a definite off-odor was apparent within 12-24 hours.

The results of various treatments on the development of black spots are presented in Table 1. Although most of the treatments caused significant decreases in the total bacterial count, only sodium bisulfite, propylene oxide and heat prevented the development of black spots. The two latter treatments gave the shrimp an abnormal pink color. Sodium bisulfite had no apparent external effect on the shrimp, yet prevented the development of spots. It had only a slight inhibitory effect on the total microbial population.

DISCUSSION

The variations in the amount and extent of black spots that develop on shrimp, coupled with ample opportunity for contamination from their natural environment and by handling, strongly suggested that these blackened areas were caused by microorganisms. The results of this investigation, however, do not support this explanation. The failure of the shrimp to develop spots when kept under anaerobic conditions as well as the prevention of blackening by dipping the shrimp in a strong reducing agent such as sodium bisulfite indicated that the discoloration was caused either by strict aerobes or was the result of some oxidative enzyme inherent in the shrimp. If the blackening were the result of the combination of H_2S produced by bacteria with some constituent of the shrimp membrane to give a black sulfide, it should have been possible to produce it by exposing fresh shrimp to an atmosphere of H_2S. The failure of this procedure to produce blackening together with the inability to isolate any microorganism producing a black pigment regardless of the medium used, the inability to demonstrate any concentration of bacteria or fungi in and around the blackened areas, the low bacterial count on some shrimp on which black spots developed, and the inability to transmit the spots to unaffected shrimp is considered to be good evidence that the black spots are not of microbial origin. Studies now under way indicate that this discoloration is actually caused by enzymes found within the shrimp. These findings will be reported at a later date.

SUMMARY

An attempt was made to find a microbial agent capable of causing black spots on fresh shrimp held in iced storage. No organisms were isolated that produced a blackening on shrimp or chitin media, and none of them caused a blackening when inoculated onto fresh shrimp. It was not possible to transmit the black spots to fresh shrimp by smear inoculation with blackened tissues. The development of spots was not affected by dipping the shrimp in many bactericidal and fungicidal agents, but it was completely inhibited by placing the shrimp under anaerobic conditions or dipping them in a strong reducing agent. It was concluded that this discoloration is not the result of microbial activity, but is caused by an oxidative enzyme of the shrimp.

Acknowledgments

The authors wish to express their appreciation to Dr. Arthur R. Colmer, Miss Carol Ernest and Miss Doris Holmes for their valuable advice and assistance in this investigation.

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TABLE 1

The effect of various treatments on the development of black spots

Treatment	Concentrations used	Time of exposure	Reduction in bacterial count	Blackening in 4-8 days
			%	
Roccal	1:500, 1:1000, 1:10,000	5 to 15 minutes	40 to 60	yes
Peracetic acid	1:100, 1:200, 1:1000	0.5 to 3 minutes	90 to 98	yes
DHA-S[f]	1:100, 1:200, 1:1000	0.5 to 3 minutes	45 to 55	yes
Sodium propionate	1:1000	15 minutes	.....	yes
Sodium bisulfite	1:100	10 minutes	30 to 50	no
Krimko Gel plus ascorbic acid	1% Krimko gel plus 0.025% ascorbic acid	1 minute	.....	yes
Propylene oxide[g]	Saturated vapor in closed jar	1 to 20 hours	98 to 99	no
Sterile water	Shrimp washed 8 to 10 times		20 to 40	yes
Heat[g]	Flowing steam	5 to 10 minutes	99	no

[f] Sodium salt of dehydroacetic acid.
[g] Shrimp developed pink color.

Techniques for Estimating the Stability of Food Products: Active Dry Yeast[a]

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Various techniques used in establishing the life expectancy of active dry yeast are described. These techniques include functional tests on both packaging materials and on the product. It is first necessary to determine the factors which affect the product. Knowing these factors, one selects packaging materials which will give adequate protection.

The factors affecting the stability or life expectancy of food products vary widely and are dependent on the character and properties of the food product in question. These products run the complete gamut of sensitivity to changes, from the highly stable products such as sugar and salt, to the highly perishable products such as fresh produce and milk. Food products, therefore, may need protection against changes in moisture content, oxygen, bacterial contamination, loss of volatile flavor and aroma constituents, browning reaction, enzyme changes, etc., in order to extend their life expectancy. The particular factors necessary to study are dependent upon the characteristics of the product under consideration.

In an investigation of the life expectancy for any given product, laboratory controlled testing is the first step. The tests should provide complete data on the qualitative and quantitative demands the product will make on its package. The tests should be so devised that the results obtained correlate with those found when the product is exposed to the conditions encountered during the distribution and merchandising of the product. Unfortunately, accelerated package studies on food products yield only relative information, since it is impossible to predict accurately the shelf life expectancy in the field on the basis of accelerated laboratory tests alone.

Discussion of the problems encountered in determining the life expectancy of food products will be limited to a consideration of the factors applicable to active dry yeast. This was selected as illustrative material for a discussion of methods since this rather unique food, although a dry product, is viable, and in order to preserve the functional activity of the product, the viability of the cells must be maintained.

In considering the problems pertinent to the shelf life of active dry yeast, let us first consider its nature and how it differs from the more familiar compressed bakers yeast or the foil wrapped yeast cake. Essentially, it is dehydrated yeast containing about 92% dry matter and 8% moisture, as compared to the 30% dry matter and 70% moisture in ordinary compressed yeast. At least 85% of the cells in active dry yeast are alive.

Active dry yeast does not represent a state of suspended animation. The yeast is respiring slowly and anything which decreases the rate of respiration without injury to the cells will have a favorable influence on the keeping quality of active dry yeast.

As mentioned before, active dry yeast has a moisture content of about 8%. As the moisture increases above this level, the stability of the product decreases. As the active dry yeast is slowly respiring, the life expectancy is significantly improved by storage in the substantial absence of oxygen, which is required for respiration. Continuous exposure to elevated temperatures of 100° F. (38° C.) or above is definitely harmful.

These characteristics indicate that the 3 most important factors influencing the life expectancy of active dry yeast are: moisture, oxygen, and temperature.

To supplement the study of these 3 packaging characteristics of active dry yeast, the moisture-vapor and oxygen permeabilities of various packaging materials were measured. Finally, the combination of the product in the package was tested both by laboratory and by field storage tests.

FUNCTIONAL TESTS

Evaluation of active dry yeast for the effect of the various factors cannot be done by chemical or physical tests. Evaluation must be made by functional tests, that is, by actual dough fermentation and baking tests. There are few, if any, known short cuts. Leavening power of the yeast is measured by the so-called punch test. The punch test is conducted as follows: a standard dough, prepared with due attention to composition, absorption, temperature, and mixing time, is placed in a graduated plastic cylinder which is placed in a constant temperature water bath or fermentation cabinet. The time required to rise to a predetermined volume is recorded, the dough is then removed from the jar, the gas expelled by running the dough through sheeting rolls and the dough replaced in the jar for another rise. With suitable attention to detail and handling procedures, this test is capable of good reproducibility. Different punch test dough formulas varying as to content of sugar and other ingredients are used to estimate the performance of the yeast in various types of bakery doughs such as bread doughs and sweet doughs.

Complete baking tests are also made in order to see how well the yeast leavens the dough and at the same time to judge the quality of the baked goods produced. As in the punch test, different dough formulas and handling procedures are used to evaluate the performance of the yeast.

The index of failure which is used for assessing the quality of active dry yeast is the change in fermentation time as measured by the punch test and by the effect on the quality of the baked goods as compared to baked goods made with compressed yeast. One of the major difficulties in evaluating active dry yeast is the lack of an authenticated standard for performance which would permit assigning a definite index of failure. At present, only arbitrary standards for acceptability are set which are maintained on the high side.

Effect of Moisture

As mentioned in the introduction, active dry yeast is very sensitive to changes in moisture. The effect of moisture changes is illustrated in Figure 1, which shows the effect on the stability of the active dry yeast when it was stored in air in a non-moisture proof package

[a] Presented at the Eleventh Annual Meeting of the IFT, New York, N. Y., June 19, 1951.

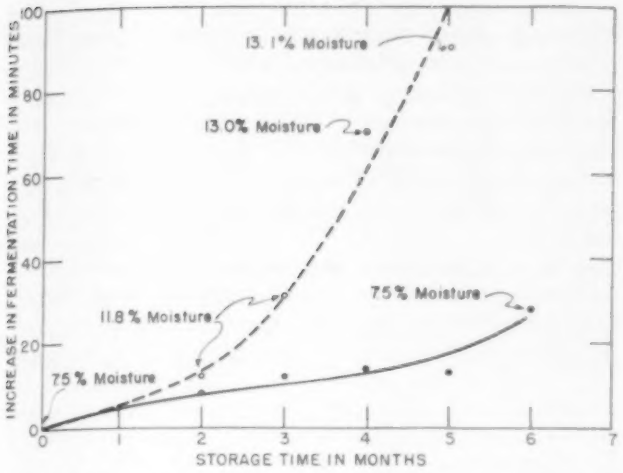

Figure 1. Effect of moisture changes on fermentation time of active dry yeast.

(dotted line) and when stored in air in a moisture proof package (solid line). As the yeast in the non-moisture proof package increased in moisture content from 7.5% to 13%, its fermenting speed decreased markedly as shown by the increase in time of first rise in the punch tests. On the other hand, the activity of the dry yeast in the moisture proof package was retained quite well for at least 5 months.

The moisture sensitivity of a food product is estimated by determination of the equilibrium relative humidity, which is one of the important product characteristics to be taken into account when preparing package specifications. Equilibrium relative humidity may be defined as that relative humidity at which a product, having a given moisture content, will neither gain nor lose moisture. For a given product the equilibrium relative humidity depends on the moisture content and the temperature.

Several methods are available for determining the equilibrium relative humidity of materials. One method is to store weighed quantities of the product in atmospheres of controlled humidity and temperature and measure the gain or loss of weight at intervals until no further change takes place. This method takes considerable time, and in the case of a living organism such as active dry yeast, respirative or other changes may cause erroneous results.

By a variation of this procedure, results can be obtained in a few hours. This method, described by Landrock and Proctor *(8)*, involves the establishment of the rate of gain or loss in weight of a sample when held in an atmosphere of constant humidity and temperature. After rate curves are determined with samples of known moisture content, it is relatively simple to evaluate unknown samples. Weighed quantities of the material are placed in a series of closed containers containing solutions of constant known humidities. The containers are held at constant temperature for 1 or 2 hours, and the material is then reweighed. If the rate of gain or loss of each sample is plotted against the humidity of the atmosphere, it is possible, by interpolation, to find the humidity at which the gain or loss would be zero, or in other words, the equilibrium relative humidity.

The method found most convenient for active dry yeast is that described by Brockington, Dorin, and Howerton *(3)*. This method employs the Aminco-Dunmore Electric Hygrometer which is similar to that described by Dunmore *(7)*. This instrument is made and calibrated by the American Instrument Company. The hygrometer consists of a humidity sensing element and micro-ammeter. To measure the equilibrium moisture content, the sensing element is submerged in the yeast which is stored in a closed container and the container placed in a water bath at the desired temperature. Humidity readings are taken at intervals until no further change in humidity is observed. The moisture content of the yeast is then determined by drying in a forced draft air oven at 100° C. overnight.

The results obtained are shown graphically in Figure 2, which shows the change in equilibrium relative humidity of active dry yeast with change in miosture content. It is seen from Figure 2 that dry yeast of 8% moisture content is in equilibrium with relative humidities of 32% to 45%, depending on the temperature, since the equilibrium relative humidity is a function of both temperature and moisture content of the sample. As will be shown later, the usual weather conditions under which the yeast is handled and sold are such that it will pick up moisture. The yeast must, therefore, be packaged in material which has the lowest possible moisture vapor permeability.

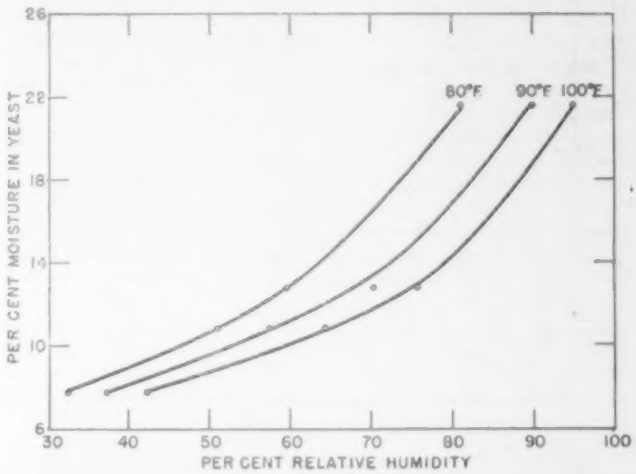

Figure 2. Equilibrium relative humidity of air in contact with active dry yeast of various moisture contents.

Effect of Oxygen

The second important packaging characteristic of active dry yeast is the effect of oxygen.

Figure 3 shows the effect of oxygen on the fermenting speed of active dry yeast when it was stored in glass jars

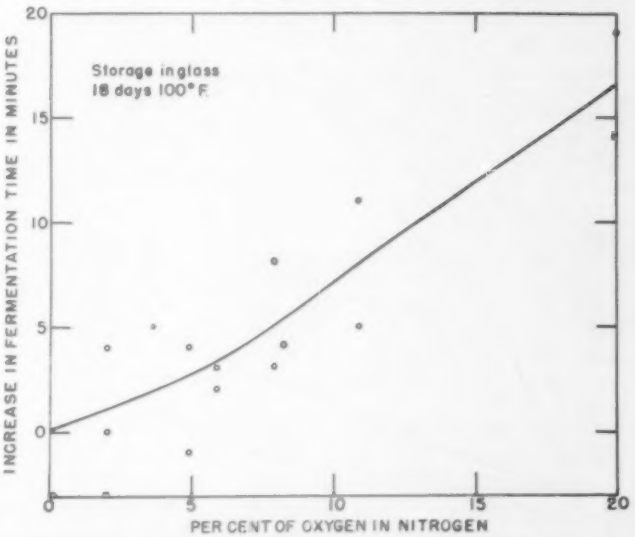

Figure 3. Effect of oxygen content of package gas on the stability of active dry yeast.

in nitrogen containing various percentages of oxygen. The increase in the punch times of the dry yeast changed more rapidly as the percentage of oxygen increased beyond 5%. The effect of oxygen is further illustrated in Figure 4, which shows the effect of storing active

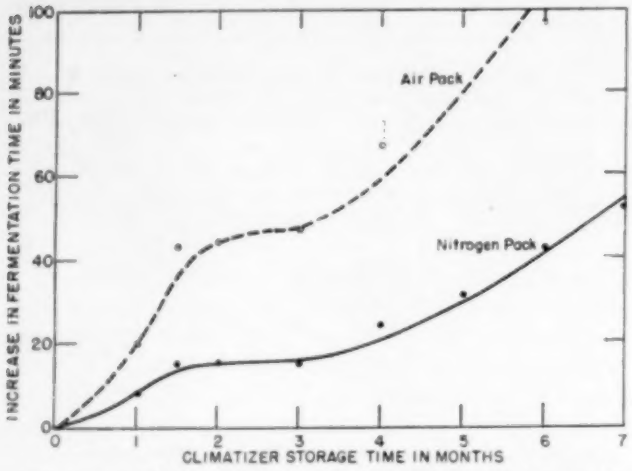

Figure 4. Effect of oxygen and nitrogen packaging on the stability of active dry yeast.

dry yeast in air and in nitrogen under conditions which did not permit of any change in moisture content. The samples packaged in air deteriorated very rapidly, while those packaged in nitrogen deteriorated slowly for the first three months, then gradually increased in rate of deterioration. These observations were first reported by Atkin, Kirby, and Frey *(2)*. The same results were confirmed later by Oyaas, Johnson and Peterson *(9)* who also found that packaging of active dry yeast in carbon dioxide, nitrogen, or vacuum, improved its stability when stored at 98.6° F. (37° C.).

Effect of Temperature

The third important factor affecting the stability of active dry yeast is temperature. Typical results are shown in Figure 5. It is seen that temperatures of 100° F. (38° C.), or higher, rapidly weaken the yeast, so that exposure to these temperatures for any length of time should be avoided. Even at refrigerator tem-

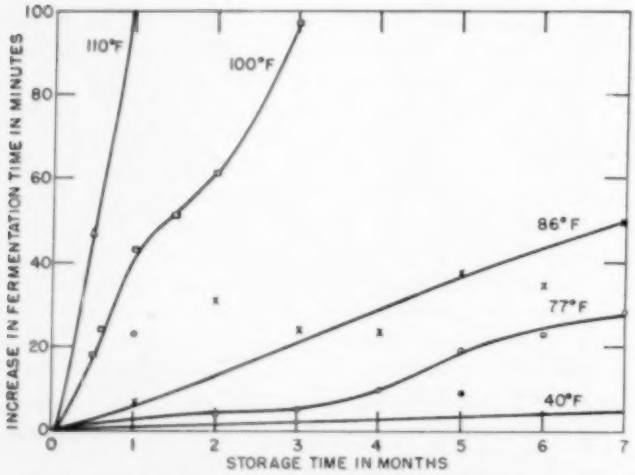

Figure 5. Effect of temperature and time of storage on the stability of active dry yeast packaged in nitrogen.

peratures, shown by the 40° F. (4.5° C.) curve, active dry yeast slowly deteriorates, but after 7 months of refrigeration it is still acceptable.

Another important consideration in regard to temperature is the fact that its effect is more a matter of the maximum temperature attained than it is of a given average temperature of storage. In other words, if a yeast is exposed for a short period to relatively high temperatures of 100° to 120° F. (38° to 49° C.), and then brought down to a lower temperature, the effect on the performance of the yeast is greater than that of exposure to the average of these 2 temperatures over the same period of time. This is illustrated in Figure 6,

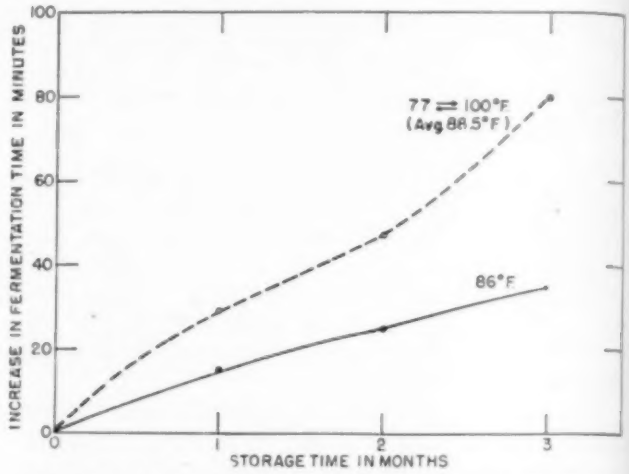

Figure 6. Effect of alternating temperatures on the storage stability of active dry yeast.

which shows the effect when nitrogen and vacuum packed samples of active dry yeast were stored at 86° F. (30° C.), constant temperature, and at an average temperature of 88.5° F., produced by alternate 12-hour cycles of 77° F. (25° C.) and 100° F. (38° C.). The slight discrepancy in average temperature will not cause this difference.

From the data presented on the packaging characteristics, it is evident that active dry yeast should be packaged in an inert gas such as nitrogen, or in vacuum, in moisture proof, gas-tight containers, and protected from elevated temperatures.

TESTS ON PACKAGING MATERIALS

After the packaging characteristics of the product have been determined, the next step is the testing of potential packaging materials.

A substantial quantity of active dry yeast is used by the Army where compressed yeast is not available. For this purpose the yeast is packed in tin cans under vacuum.

A principal use of active dry yeast is in the household, where it replaces the familiar small cakes of compressed yeast. It is desirable that each unit package of active dry yeast be equivalent to 1 cake of compressed yeast so that the usual baking recipes will not be disturbed. Small flat pouches of flexible material are used as the unit package because of ease of handling, and because machines are available for this type of package.

The 2 most important properties of a packaging material for use with active dry yeast are its moisture vapor transmission and its oxygen permeability.

Various methods have been proposed for the determination of moisture vapor transmission (MVT). The A. S. T. M. *(1)* lists 2 procedures: one for relative humidities of 50%, and another for humidities near 90% at 100° F. (38° C.). Doty, Aiken, and Mark *(6)* described a method suitable for films of very low MVT. A modification of this has also been published by Doty *(5)*.

The method most generally used by manufacturers and users of paper products appears to be the General Foods Moisture Vapor Test Cabinet and procedure *(11)*. The method in brief consists in measuring the gain in weight of a dish containing anhydrous calcium chloride, the cover of which is a sheet of the material under test. Exposure conditions are 90% relative humidity at 100° F. (38° C.).

Various methods have been proposed for the determination of oxygen permeability. Some of these employ a pressure gradient across the test specimen, while others are isostatic. The flexible packets used for active dry yeast have an internal pressure essentially equal to atmospheric pressure, so that isostatic methods are preferable. For vacuum packaging materials, methods involving pressure differences might give more useful results.

One oxygen permeability method tried was that of Davis *(4)*. In this method a number of sheets of the material to be tested are stacked together in a filter press type apparatus. Oxygen is flowed past one side of the material, and the amount of oxygen which diffuses through is swept out with a stream of carbon dioxide flowing past the other side. The carbon dioxide is then absorbed in caustic solution, and the oxygen collected and measured. The method, although cumbersome and time-consuming, appears to give reliable results.

Another method is described by Zambito *(12)* which can be used for sheet materials having very low oxygen permeabilities. This method depends on the color produced on the filament of an electric bulb when burned at 15 volts in an atmosphere containing from 10 to 150 p.p.m. of oxygen. Colors range from light tan through blues and reds to black.

In addition to having low permeabilities to moisture and oxygen, it is desirable that the packaging material be suitable for fabrication into pouches on commercially available packaging machinery. Trial runs quickly establish the suitability of the packaging material. It may be necessary, of course, to design special machinery if a desirable type of packaging material cannot be used with available equipment. For active dry yeast 3- or 4-ply laminates of pliofilm, aluminum foil, and paper have been successfully used.

Tests on Sealed Packages

After the packaging characteristics of the product and the characteristics of the packaging material have been determined, the final test covers the characteristics of the packaged product. These represent a composite of the packaging material itself and the formation of the package by the machine which performs some type of sealing operation.

The 2 tests most used on sealed packages are the determination of leaks and the oxygen content of the gas in the package.

One of 2 methods is used to determine leaks in packages. In one, the package is immersed in water in a vacuum desiccator. Vacuum is applied and the packages are checked visually for leaks. The other is used in conjunction with the oxygen determination and will be described below.

For the determination of oxygen, 2 general procedures are used. In one of these, a measured volume of the gas from a packet immersed in oil or water is withdrawn by means of a hypodermic syringe, and the gas is introduced through a serum bottle stopper into a conventional gas analysis apparatus, such as the Orsat, and analyzed.

The most rapid method employs the Beckman oxygen analyzer which depends on the paramagnetic properties of oxygen and was designed by Pauling, Wood, and Sturdivant *(10)*. In using the Beckman instrument either the hypodermic syringe technique as for the Orsat method, or a low vacuum method is used. The latter method is more rapid but less precise than the syringe method. In the syringe method the gas is withdrawn by hypodermic syringe under liquid to prevent atmospheric contamination and introduced into the oxygen analyzer through a serum bottle stopper. The newly introduced sample flushes out the previous sample, the instrument cell volume having approximately 3 ml. capacity, and the analyzer reads the oxygen concentration directly.

In the low vacuum method, the hypodermic needle is attached to the tubing of one opening of the oxygen analyzer. The other opening is connected to a manometer and a mercury column and leveling bulb. A small patch of rubber is cemented to the package so that there will be no leakage around the needle when it is pushed through the patch into the package. The needle is inserted into the package through the patch and the leveling bulb is lowered. Gas is drawn from the package into the analyzer. When pressure equilibrium is reached, the analyst records the manometric pressure in the system and the reading of the oxygen analyzer. High oxygen values indicate poor initial packaging or leaks in the package. Failure of the manometer to maintain equilibrium indicates a leak. It is convenient to have the oxygen analyzer calibrated in partial pressure rather than in percent oxygen. Then from these 2 readings it is possible to calculate the percentage of oxygen in the gas.

LABORATORY AND FIELD STORAGE TESTS

Two kinds of laboratory storage tests were conducted. In one of these the packaging characteristics of the active dry yeast were measured independently of the packaging material. These storage tests were made with flame-sealed, spherical shaped glass flasks or with glass jars sealed with gasketed metal caps in a single bell White Cap sealing machine. Air in the container was replaced by evacuating the system, then admitting nitrogen or other inert gas and sealing. Glass packaging was used in those cases in which it was desired to remove the failure of the packaging material as a factor, such as in the case of extended storage at various temperatures.

In the second type of test, the yeast was stored in the type of packaging material under test. These materials included various combinations of foil, plastic, and treated paper.

The various samples in "glass" or in packets, were stored in conventional laboratory incubators and the active dry yeast was tested after storage for the desired length of time. For tests with an alternating temperature and humidity, an Aminco Climatizer was used. This unit consists of a humidity and temperature controlling cabinet conected to a storage cabinet. Two sets of temperature and humidity controls were installed which were selected by a time clock for alternate cycling between pairs of selected temperatures and relative humidities. The conditions used were 90°F. (32° C.) and 67% relative humidity for 12 hours, followed by 76° F. (24.5° C.) and 90% relative humidity for 12 hours.

To further check the laboratory tests, field tests were carried out on the packaged yeast. The field locations selected were: Jacksonville, Florida; New Orleans, Louisiana; and Tucson, Arizona. Southern locations were used, since, when the dry yeast is properly packaged, the temperature at which it is held is a principal factor affecting stability.

A comparison between active dry yeast stored in the Climatizer, and at various field locations was also made. Some of the results are shown in Figure 7. It is seen that the samples which were stored in the Climatizer deteriorated more gradually than those stored at the field locations. Samples stored in the field locations

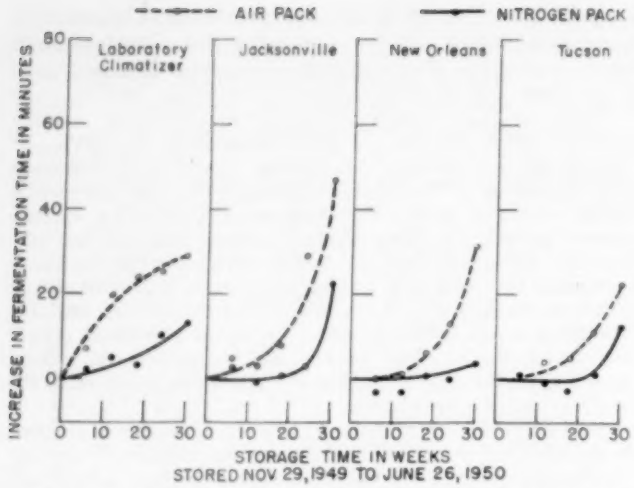

Figure 7. Comparison of laboratory and field storage tests of active dry yeast.

retained fermenting speed until the warmer months of the spring and early summer were reached, at which time, as would be expected from the laboratory tests, they lost fermenting speed. Thus, even though active dry yeast is properly packaged, it requires care in handling and distribution to have it reach the consumer in good condition. The data shown in Figure 7 again indicate the advantage of packaging in nitrogen.

SUMMARY

Data have been presented showing the techniques used in establishing the life expectancy of active dry yeast. These techniques include functional tests on both packaging materials and on the product. These techniques can be applied to almost any food product.

It is first necessary to determine the factors which affect the product. In the case of active dry yeast, the 3 most important factors are exposure to moisture, oxygen, and high temperature. Knowing these factors, packaging materials are selected which will give adequate protection against moisture, and then the product is packaged in an inert gas to prevent the deleterious effects of oxygen.

Although the methods and data presented in this paper have been limited to a consideration of active dry yeast, the following general procedure may be applied for estimating the stability of any good product:

From a consideration of the product, it should be possible to determine the factors which will influence its stability. After selection of a method or means of evaluating deterioration, laboratory measurement of the packaging characteristics of the product should be made. On the basis of these findings, suitable packaging material can be selected to give the necessary protection. In making these selections laboratory tests are of prime importance because they can be performed under controlled and reproducible conditions. However, the final evaluation of the stability of the product must be done with the aid of actual field tests.

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Effect of Antibiotics in Retarding the Growth of Micrococcus pyogenes var. aureus in Custard Fillings

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Of the antibiotics employed, only subtilin demonstrated a 2-fold effectiveness of controlling both the general heat-resistant spoilage flora in custard fillings and the food-poisoning micrococci that were experimentally added to these fillings. The combination of terramycin with subtilin resulted in very good preservative control. Aureomycin and terramycin used independently in trace amounts were very effective against the pathogenic food-poisoning micrococci but not against the heat-resistant spoilage flora. Penicillin was less effective than either aureomycin or terramycin against the pathogenic organisms and showed very slight sporicidal activity. Bacitracin, chloromycetin, and streptomycin were valueless in the concentrations used.

Custard pastry fillings often have been implicated in outbreaks of food poisoning *(10, 11)*. Certain enterotoxin-producing strains of *Micrococcus pyogenes* var. *aureus* are often found to be the responsible agents for producing the toxins in these products. The ubiquity of these food-poisoning micrococci has made it mandatory that custard-filled baked goods be prepared, stored, and handled under rigidly sanitary conditions.

Besides the custard-filled baked goods, there are many other pastry fillings which support the growth of food-poisoning micrococci *(8)* and which do not come under regulatory laws of preparation and handling. With many of these baked items there is less personal handling, but they still can be a potential source of food poisoning, if contaminated.

During the past 2 years there have been numerous investigations as by Andersen and Michener *(2)*, Adams, Ayres, and Tischer *(1)*, Bohrer *(4)*, Burroughs and Wheaton *(5)*, Cameron *(6)*, Cameron and Bohrer *(7)*, Olcott *(15)*, Tarr, Southcott, and Bissett *(17)*, and Williams and Campbell *(18)*, for instance, with the use of antibiotics for the preservation of canned foods and the retardation of fish spoilage. In the preservation of canned foods the early success of subtilin and mild heat reported by Andersen and Michener *(2, 3)* was later found to be of limited value or of very slight promise by other investigators. Cameron and Bohrer *(7)* have summed up these investigations with subtilin and other antibiotics by saying that there is no antibiotic at present which can be used as a substitute for high temperature processing. Tarr, Southcott, and Bissett *(17)* found aureomycin and terramycin to be effective for retarding fish spoilage at low temperature storage of 33° to 37° F. (0.5° to 3° C.) for 7 to 10 days. These same authors stated that fairly good preservation was obtained with chloromycetin, weak preservation with penicillin and streptomycin, and that subtilin was completely valueless in this respect.

Owing to the short shelf-life of a custard-filled product, it was considered to be of interest to see how long preservation against food-poisoning micrococci and the natural heat-resistant flora of such a filling could be retarded when stored at a temperature which would be most favorable for their growth. A few exploratory tests with several antibiotics and food poisoning strains of micrococci gave us some idea of the sensitivity of these organisms to the antibiotics used. It was decided, therefore, to test these antibiotics in prepared fillings with experimentally added food-poisoning micrococci.

EXPERIMENTAL

Physiological Characteristics of Test Organisms

Four strains of *Micrococcus pyogenes* var. *aureus* from varying sources were used in this experimental work. The physiological characteristics of these strains are shown in Table 1. The basal fermentation media and nutrient gelatin were similar in composition and preparation to those described by Evans *(12)* and Evans and Niven *(13)*. Based on these data (Table 1) it suffices to say that the 4 strains fall into a rather homogeneous group and indicate that they could produce food poisoning.

Procedures

The following custard filling formula was used in this work and differed only from the formula of Cathcart, Merz, and Ryberg *(9)* in the substitution of non-fat dry milk solids for powdered whole milk.

	Pounds	Ounces
Water	2	....
Sugar	....	8
Non-fat dry milk solids	....	4
Cornstarch	....	2.5
Eggs (whole)	....	3
Salt	....	⅛

In the preliminary experiments 50-g. portions of the prepared custard filling were carefully weighed out into small wide-mouth bottles 2½ inches wide × 3¼ inches high (outer diameter of mouth approximately 2 inches). The bottles containing the fillings were covered with aluminum foil and then autoclaved at 15 p.s.i.g. for 10 minutes. After cooling, measured amounts of each antibiotic [a] from freshly prepared concentrates were added and carefully stirred into the fillings under aseptic conditions. The remaining procedure of adding the test organisms and subsequent incubation and plating was essentially the same as that described in Cathcart, Godkin, and Barnett *(8)*.

[a] Subtilin of 70% potency was obtained from Dr. H. S. Olcott of the Western Regional Research Laboratory, Albany, California. The other antibiotics used were purchased from commercial sources.

TABLE 1

Physiological characteristics of test organisms (Micrococcus pyogenes var. aureus)

Characteristics	Strain NRRL-B-678	Strain NRRL-B-314	Strain No. 161	Strain No. 49
	Food poisoning [a]	Penicillin testing [a]	Food poisoning [b]	Finger infection. Stock culture
Pigment formation—Staphylococcus medium No. 110 and Chapman Stone medium	Orange	Cream	Orange	Orange
Hemolysis	Positive	Positive	Positive	Positive
Coagulase	Positive	Positive	Positive	Positive
Stone's reaction—Chapman Stone medium	Negative	Positive	Positive	Positive
Nutrient gelatin hydrolysis	Negative	Positive	Positive	Positive
Fermentation:				
Mannitol, aerobic [c]	Acid	Acid	Acid	Acid
Mannitol, anaerobic	Acid (slow)	Acid	Acid	Acid
Sucrose, aerobic	Acid	Acid	Acid	Acid
Sucrose, anaerobic	Acid	Acid	Acid	Acid
Dextrose, aerobic	Acid	Acid	Acid	Acid
Dextrose, anaerobic	Acid	Acid	Acid	Acid
Lactose, aerobic	Acid	Acid	Acid (slow)	Acid (slow)
Lactose, anaerobic	Acid	Acid	Acid (slow)	Acid (slow)
Glycerol, aerobic	Acid	Acid	Acid	Acid
Glycerol, anaerobic	Slight Acid	Slight Acid	Slight Acid	Slight Acid
Litmus milk reactions:				
Acid	Positive	Positive	Positive (slow)	Positive (slow)
Curd	No	No	No	No
Proteolysis	No	No	No	No

[a] NRRL—Northern Regional Research Laboratory, Peoria, Illinois. Courtesy of Dr. W. C. Haynes.
[b] Courtesy of Dr. G. M. Dack, Professor of Bacteriology, University of Chicago.
[c] Mannitol fermentations on Staphylococcus Medium No. 110 and Chapman Stone medium were very weak and difficult to evaluate.

Tryptone Glucose Extract Agar (Difco) was used with the autoclaved fillings as the standard plating medium. In later experiments the fillings were not autoclaved and Staphylococcus Medium No. 110 (Difco) was used with Tryptone Glucose Extract Agar.

The procedure was slightly altered with the non-autoclaved fillings. The antibiotics were dry-mixed with the cornstarch ingredient so that they could be added during the normal preparation of the custard filling. By varying the amount of (cornstarch plus antibiotic) with pure cornstarch during the preparation of the filling, the desired amount of antibiotic could readily be maintained in the filling without altering the cornstarch as per formula. These fillings were not autoclaved, but the small wide-mouth sampling bottles were sterilized before filling and all transferring was done under aseptic conditions. The test organisms were added to the sample bottles containing the normally prepared cooled fillings. With this method of preparation each antibiotic was subjected to heat as the mixture containing the antibiotic was brought to a boil and held there for 1 minute before cooling.

The coagulase tests shown in Table 4 were performed by taking a small loopful of the filling (about ⅛ inch in diameter) and emulsifying it with 0.25 ml. of Brain Heart Infusion Broth (Difco). To this emulsion 0.5 ml. of fresh rabbit plasma was added, mixed thoroughly, and incubated at 98.6° F. (37° C.). Similar coagulase tests also were conducted using (Bacto) desiccated coagulase plasma.

Throughout this experimental work counts were made with the aid of a Greenough binocular wide-field microscope under both ×7 and ×15 magnifications. The colonies were carefully examined to see that they conformed morphologically to the colony type of the strain used. Further examinations were made by preparing stained smears on a slide and examining the cells at ×1000. The coagulase test was also utitlized in checking these colonies.

RESULTS

The results of the preliminary experiments with autoclaved fillings, listed in Table 2, show that trace amounts of aureomycin and terramycin (0.6 p.p.m. to 1.0 p.p.m.) were most effective against *M. aureus* during a 24-hour incubation of the fillings at 98.6° F. (37° C.). Penicillin showed slight effectiveness at a concentration of 1.2 p.p.m. Bacitracin and chloromycetin were valueless at concentrations of 40-60 p.p.m. and 4-5 p.p.m., respectively. Against the NRRL-B-678 strain 5 p.p.m. of streptomycin was slightly inhibitory. This same amount of streptomycin had no effect against strain No. 161. Subtilin demonstrated inhibitory action against *M. aureus* at a concentration of 10 p.p.m. and bactericidal action at 20 p.p.m. concentration.

TABLE 2

Effect of several antibiotics against Micrococcus pyogenes var. aureus in autoclaved custard fillings

Antibiotic	Concentration	Strain inoculated	Organisms added per g. filling	Organisms per g. filling 24 hr. later
	p.p.m.			
Penicillin	0.96	161	95,000	13,000,000
Penicillin	1.20	161	17,000	110,000
Bacitracin	40.0	161	95,000	1,100,000,000
Bacitracin	60.0	161	17,000	410,000,000
Streptomycin	4.0	161	95,000	900,000,000
Streptomycin	5.0	161	17,000	35,000,000
Streptomycin	5.0	NRRL-B-678	78,000	700,000
Chloromycetin	4.0	161	95,000	850,000,000
Chloromycetin	5.0	161	17,000	150,000,000
Aureomycin	1.0	161	17,000	< 500
Aureomycin	0.8	161	17,000	6,000
Aureomycin	0.8	161	95,000	220,000
Aureomycin	0.6	161	17,000	3,000
Aureomycin	0.6	NRRL-B-678	78,000	21,000
Terramycin	0.8	161	95,000	25,000
Terramycin	0.8	161	17,000	< 500
Terramycin	0.6	161	17,000	< 500
Terramycin	0.6	NRRL-B-678	78,000	< 500
Terramycin	0.4	161	17,000	30,000
Subtilin	20.0	161	95,000	25,000
Subtilin	20.0	161	17,000	11,000
Subtilin	20.0	161	34,000	1,000
Subtilin	10.0	161	17,000	81,000
Subtilin	5.0	161	17,000	12,000,000
Subtilin *	20.0	NRRL-B-678	78,000	< 500
Subtilin	20.0	NRRL-B-678	78,000	< 500
None (Control)		161	93,000	2,600,000,000
None (Control)		161	17,000	630,000,000
None (Control)		NRRL-B-678	78,000	26,000,000

* Sample containing subtilin heated at 212° F. for 1 minutes, not autoclaved.

The results with the non-autoclaved fillings are shown in Tables 3 and 4. Table 3 lists results during a 24-hour incubation of the custards and here it is shown that 1.0 p.p.m. concentrations of aureomycin and terramycin were effective against *M. aureus*. However, neither of these antibiotics demonstrated any control against the heat-resistant contaminating bacilli and the fillings spoiled readily.[†] These heat-resistant contaminating organisms were originally bacterial spores present in the ingredients and could be considered as the natural flora of the prepared custard fillings. In 2 cases the contaminant growth was so great on both plating media that it was impossible to obtain counts of *M. aureus*. Penicillin in concentration of 3 p.p.m. was slightly inhibitive to *M. aureus* (in comparison to control count) but showed very little sporicidal effect. Subtilin was effective against both *M. aureus* and the heat-resistant spoilage organisms. No spoilage of the fillings was noticed during 24 hours of incubation at 98.6° F. (37° C.) when 50 p.p.m. and 100 p.p.m. of subtilin were used. In 2 cases slight spoilage occurred when 25 p.p.m. of subtilin was used.

Up to this point subtilin had demonstrated the greatest activity against the heat-resistant spoilage organisms which comprised the natural flora of the fillings. Consequently most of the work was centered upon this antibiotic. Table 4 lists results showing the effectiveness of subtilin where the incubation of the custard fillings at 98.6° F. (37° C.) was carried beyond 24 hours. For the sake of brevity only the highest *M. aureus* count obtained from either plating medium is shown in this table. On the average, counts ran slightly higher on the Tryptone Glucose Extract Agar but in many cases compared favorably on both media. In several cases 25 and 50 p.p.m. of subtilin did not retard the growth of *M. aureus* for more than 24 hours. These same concentrations of subtilin delayed spoilage to some extent but results varied and no doubt were dependent on the type and number of heat-resistant flora originally present in the filling. In a filling devoid of contaminating organisms, 50 p.p.m. of subtilin retarded the growth of *M. aureus* for 5 days.

Increasing the antibiotic concentration to 100 p.p.m. resulted in a greater protection against *M. aureus* activity over a 72 hour holding period. With one of these samples slight spoilage was noticed within 72 hours. The coupling of 1.0 p.p.m. of terramycin along with 100 p.p.m. of subtilin had a very pronounced effect against

[†] Spoilage by contaminants as expressed in this paper varied from a slightly sour aroma to an odor of putrefaction and was accompanied by a breakdown in the consistency of the filling. *M. aureus* does not alter the appearance or produce any "off" odor in the filling.

TABLE 3

Effect of several antibiotics against Micrococcus pyogenes var. aureus in non-autoclaved custard fillings

Antibiotic	Concentration	Strain inoculated	Organisms added per g. filling	Organisms per g. filling 24 hours later		Remarks
				Tryptone gl. ex. ag.	Staph. med. 110	
	p.p.m.					
Aureomycin	0.6	161	2,000	No count	No count	Spreader growth. Filling spoiled.
Aureomycin	1.0	NRRL-B-314	9,000	< 500	< 500	Filling spoiled by contaminants.
Terramycin	0.6	161	2,000	No count	No count	Spreader growth. Filling spoiled.
Terramycin	1.0	NRRL-B-314	9,000	< 500	< 500	Filling spoiled by contaminants.
Penicillin	3.0	NRRL-B-314	9,000	No count	1,200,000	Filling spoiled. Sporicidal effect minimal.
Subtilin	20.0	161	2,000	40,000	38,000	No spoilage.
Subtilin	10.0	161	2,000	600,000	540,000	Same.
Subtilin	25.0	NRRL-B-314	9,000	500	300	Same.
Subtilin	50.0	NRRL-B-314	9,000	8,900	6,900	Same.
Subtilin	25.0	NRRL-B-678	150,000	16,000	33,000	Same.
Subtilin	25.0	161	180,000	105,000	92,000	Slight spoilage. Partial sporicidal effect.
Subtilin	25.0	49	33,000	55,000	45,000	Same.
Subtilin	50.0	NRRL-B-678	63,000	6,000	4,000	No spoilage. Heat-resistant organisms retarded.
Subtilin	50.0	NRRL-B-314	62,000	155,000	160,000	Same.
Subtilin	50.0	161	17,000	36,000	32,000	Same.
Subtilin	50.0	49	85,000	60,000	90,000	Same.
Subtilin	25.0	161	314,000	3,600,000	2,900,000	Heat-resistant organisms propagating. No spoilage noted.
Subtilin	50.0	161	314,000	20,000	19,000	Same.
Subtilin	100.0	161	314,000	57,000	58,000	No spoilage. Heat resistant organisms retarded.
Subtilin	100.0	161	266,000	51,000	49,000	Same.
Subtilin and Terramycin	50.0 } 1.0 }	161	17,000	100	100	Same.
Subtilin and Terramycin	100.0 } 1.0 }	161	266,000	800	1,000	Same.
None (Control)		161	2,000		500,000,000	Growth of contaminants unrestricted. Filling very spoiled.
None (Control)		NRRL-B-314	9,000	560,000,000	680,000,000	Same.
None (Control)		NRRL-B-678	150,000	410,000,000	300,000,000	Same.
None (Control)		161	180,000	3,200,000,000	2,200,000,000	Same.
None (Control)		49	33,000	570,000,000	485,000,000	Same.
None (Control)		NRRL-B-678	63,000		189,000,000	Same.
None (Control)		NRRL-B-314	62,000		348,000,000	Same.
None (Control)		161	17,000		266,000,000	Same.
None (Control)		49	85,000		430,000,000	Same.
None (Control)		161	314,000	188,000,000	130,000,000	Same.
None (Control)		161	266,000	640,000,000	730,000,000	Same.

M. aureus. After 72 hours of incubation the *M. aureus* count was less than 100 organisms per g. of filling and, following 8 days of incubation, a count of 62,000 *M. aureus* organisms was obtained. Spoilage was not observed in this sample during the 8 day holding period.

DISCUSSION

Although very small amounts of aureomycin and terramycin (0.6 to 1.0 p.p.m.) were effective against *M. aureus*, the use of these antibiotics in such trace amounts is limited because of their ineffectiveness against the natural heat-resistant spoilage organisms present in the custard fillings. We do not know at present if higher levels of these antibiotics would have a sporicidal effect against the contaminants. The same can be said for penicillin with respect to the spoilage flora and which in addition was less effective against *M. aureus* than either aureomycin or terramycin. In the concentrations used, bacitracin (40 and 60 p.p.m.), chloromycetin (4.0 and 5.0 p.p.m.), and streptomycin (4.0 and 5.0 p.p.m.) did not show any promise in a custard filling containing only *M. aureus* organisms. With these latter antibiotics higher concentrations were not resorted to since their effect at the concentrations used in no way compared to the antibiotic activity of aureomycin and terramycin. Of all the antibiotics studied, only subtilin demonstrated a controlling action against both the *M. aureus* organisms and the natural heat-resistant flora of the fillings.

Subtilin was more effective against *M. aureus* when the natural heat-resistant flora were eliminated from the custard filling. Although assays were not determined on any of the incubated non-autoclaved fillings it is presumed that part of the subtilin was destroyed by heat when preparing the custard fillings. Also it is possible that some of the heat-resistant contaminants utilized the subtilin. Burroughs and Wheaton *(5)* found a considerable loss of potency of the antibiotic subtilin toward *B. stearothermophilus* after this antibiotic was assayed from spoiled cans of peas containing both A and B strains of *Cl. botulinum*. In 2 cases (see Table 4) there were examples of the *M. aureus* organisms acquiring a resistance to subtilin when in contact with this antibiotic for an appreciable time (8 to 10 days). No spoilage was noticed in these fillings and the contaminating flora were practically nil. In many instances the rise in the *M. aureus* count after longer incubation of the fillings was also accompanied by spoilage (Table 4). However, several cases were observed where a contaminant grew fairly well without producing a spoilage aroma or breakdown in the consistency of the filling. In such instances there was a rise in the *M. aureus* count on longer incubation of the filling (48-72 hrs.). Some of the fillings containing 25 p.p.m. of subtilin were very slightly spoiled within 24 hours. However, all of the control fillings which did not contain subtilin were very spoiled in 24 hours and also showed very high *M. aureus* counts. Thus it is shown that subtilin had a retarding effect on both the heat-resistant flora and the *M. aureus* organisms during the first 24 hours of incubation of the fillings. This effect was maintained over a longer incubation period when the concentration of subtilin was increased to 100 p.p.m. The use of 2 antibiotics in the same filling (subtilin 100 p.p.m. and terramycin 1.0

TABLE 4

Prolonged effect of subtilin against Micrococcus pyogenes var. aureus and contaminants in non-autoclaved custard fillings

Antibiotic	Concentration	Strain inoculated	Organisms added per g. filling	Organisms per g. filling for incubation time as shown				Coagulase test [a]	Remarks
				24 hours	48 hours	72 hours	As shown		
	p.p.m.								
Subtilin	25	NRRL-B-314	9,000	500	3,000	.....	.....	N. T.	No spoilage in 48 hours.
Subtilin	50	NRRL-B-314	9,000	8,900	<100	.....	*10 days:* 800,000,000	N. T.	No spoilage in 10 days. Absence of contaminating flora.
Subtilin	25	NRRL-B-678	150,000	33,000	.....	30,000,000	.....	(—) 6 hr.	No spoilage.
Subtilin	25	161	180,000	105,000	.....	3,000,000,000	.....	(+) 1½ hr.	Very slight spoilage within 24 hours.
Subtilin	25	49	33,000	55,000		336,000,000	.....	(+) 1½ hr.	Same.
Subtilin	50	NRRL-B-678	63,000	6,000	.....	150,000	.....	N. T.	Slight spoilage within 48 hours.
Subtilin	50	NRRL-B-314	62,000	160,000	.....	880,000,000	.....	N. T.	Definitely spoiled in 48 hours.
Subtilin	50	161	17,000	36,000	.....	30,000,000	.....	N. T.	Slight spoilage in 72 hours.
Subtilin	50 [b]	161	17,000	*5 days:* 28,000			*10 days:* 1,200,000,000	N. T.	No spoilage in 10 days.
Subtilin	25	161	314,000	3,600,000	112,000,000	226,000,000	.....	(+) 3 hr.	No spoilage within 72 hours.
Subtilin	50	161	314,000	20,000	1,700,000	72,000,000	.....	(+) 4 hr.	Same.
Subtilin	100	161	314,000	58,000	34,000	3,000	.....	(—) 6 hr.	Same.
Subtilin	100	161	314,000		38,000	294,000	.....	(—) 6 hr.	Same.
Subtilin	100	161	266,000	51,000	68,000	2,000,000	*8 days:* 253,000,000	(+) 3 hr.	Slight spoilage within 72 hours.
Subtilin and Terramycin	100 } 1 }	161	266,000	1,000	<100	<100	.....	(—) 6 hr.	No spoilage within 72 hours.
Subtilin and Terramycin	100 } 1 }	161	266,000		7,200	<100	*8 days:* 62,000	(—) 6 hr.	No spoilage within 8 days.
None (Control)		NRRL-B-314	9,000	680,000,000	.....	.....	.....	N. T.	Very spoiled in 24 hours.
None (Control)		NRRL-B-678	150,000	410,000,00	.....	.....	.....	(+) 6 hr.	Same.
None (Control)		161	180,000	3,200,000,000	.....	.....	.....	(+) 1 hr.	Same.
None (Control)		49	33,000	570,000,00	.....	.....	.....	(+) 1 hr.	Same.
None (Control)		NRRL-B-678	63,000	189,000,000	.....	.....	.....	N. T.	Same.
None (Control)		NRRL-B-314	62,000	348,000,000	.....	.....	.....	N. T.	Same.
None (Control)		161	17,000	261,000,000	.....	.....	.....	N. T.	Same.
None (Control)		161	314,000	188,000,000	.....	.....	.....	(+) 3 hr.	Same.
None (Control)		161	266,000	730,000,000	.....	.....	.....	(+) 2 hr.	Same.

[a] Coagulase test: N.T. indicates No test; (+) 1½ hr. indicates Positive in 1½ hr.; (—) 6 hr. indicates negative in 6 hr.; tests refer to time when highest *M. aureus* count is shown.

[b] Filling intermittantly sterilized prior to adding *M. aureus* organisms.

p.p.m.) was very effective against controlling the growth of *M. aureus* and also preventing spoilage of the filling.

It is the authors' view that the antibiotic effect of subtilin might be more pronounced in custard fillings when stored under average room temperature conditions. In this particular report we were interested in testing the effects of subtilin and the other antibiotics under temperature conditions which most favored the normal heat-resistant flora and the experimentally added *M. aureus* organisms. Under average room temperature storage it is believed that the addition of 100 p.p.m. of 70% potency subtilin to a custard filling will retard the growth of food-poisoning micrococci and heat-resistant spoilage organisms normally present in a filling for a period of 3 days. A stronger preservative effect may be obtained by incorporating a small amount (1.0 p.p.m.) of terramycin or aureomycin along with 100 p.p.m. of 70% potency subtilin.

It is not known what effect the storage of fillings at 98.6° F. (37° C.) had on the subtilin potency. Bohrer *(4)* found that the loss of subtilin potency was more pronounced at 131° F. (55° C.) than at 98.6° F. (37° C.). Consequently future work with this antibiotic in custard fillings will also include microbiological assay to determine the probable loss in potency during storage.

SUMMARY

The growth of food-poisoning strains of *Micrococcus pyogenes* var. *aureus* in custard fillings incubated at 98.6° F. (37° C.) was retarded for 48 to 72 hours by the addition of 100 p.p.m. of 70% potency subtilin to the filling. Spoilage caused by the natural heat-resistant flora of the filling was also retarded for 48 to 72 hours under the same conditions of storage and concentration of subtilin. A greater preserving effect against both the pathogenic and non-pathogenic organisms was obtained when 1.0 p.p.m. of terramycin was combined with 100 p.p.m. of 70% potency subtilin in the filling.

Small amounts of aureomycin and terramycin, (0.6 to 1.0 p.p.m.) very effectively retarded the growth of *M. aureus* organisms in a custard filling but did not demonstrate any control against the non-pathogenic heat-resistant flora responsible for spoilage. Penicillin was less effective against *M. aureus* than either aureomycin or terramycin and showed very little sporicidal

activity. Bacitracin, chloromycetin, and streptomycin were valueless in the concentrations used.

On the basis of results obtained at 98.6° F. (37° C.) incubation it is believed that the addition of 100 p.p.m. of 70% potency subtilin will preserve the custard filling against food-poisoning micrococci and the heat-resistant non-pathogenic spoilage organisms for a period of 3 days at normal room temperature storage. This preserving effect can be further augmented by combining a small amount (1.0 p.p.m.) of terramycin or aureomycin along with the 100 p.p.m. concentration of subtilin.

USE OF ANTIBIOTICS IN CUSTARD FILLINGS[1]

It is realized that the use of antibiotics as food preservatives necessitates that complete knowledge of their long-term toxicity effects be known. Some of the reports of toxicity studies *(14, 16, 19)* would indicate that the most promising antibiotics cited in this report are relatively non-toxic. However, the term of these studies range only from 2 to 7 months. It must be emphasized therefore that more information of feeding tests of a longer duration is needed.

[1] (EDITOR'S NOTE: The physiological effects of various antibiotics, if used in foods, has not been clearly established. Practical use of these agents in foods is not permitted at the present time.)

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Preparation of Full-Flavored Apple Juice with Increased Resistance to Fermentation

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The use of ion-exchange resins for increasing the resistance of apple juice towards fermentation has been investigated. A single passage through a cation exchange resin alone has been found to confer a high degree of resistance to fermentation provided that only 6 volumes of juice per volume of resin bed are demineralized. There is considerable loss of volatile flavor by this treatment, and sugar content and acidity are also affected. A full-flavored juice of high fermentation resistance can be prepared if the volatile flavor is removed before demineralization and subsequently returned to the juice after suitable adjustment of the sugar content and acidity.

After removal of the closure from a bottle or can of pasteurized apple juice, the contents are readily susceptible to fermentation as a result of external yeast infection. Since it is necessary to consume the juice within 2 or 3 days after opening the container, attempts to market apple juice in Britain in containers of 26 fluid-ounce or of larger capacity have not proved so popular among consumers as the small size packs. Although the onset of fermentation can be delayed by addition of sulfur dioxide or sodium benzoate, the high dosage required is readily discernible to the palate and the delicate flavor and bouquet of the juice is marred.

Challinor, Kieser, and Pollard *(3)* found that apple juice could be rendered more stable against fermentation by treatment with ion exchange resins. After passage of juice from Rival apples through a column of Zeo-karb HI, followed by a column of De-Acidite B and finally through a second column of Zeo-karb HI, the ash content of the juice was reduced to 1% of its original value and the total nitrogen fell to 1.2 mg. per 100 ml. compared with 13.6 mg. in the original juice. Storage of the unpasteurized demineralized juice at laboratory temperature without adopting aseptic precautions showed no obvious fermentation after 4 weeks compared with the untreated juice which rapidly fermented within 3 days. They stated that the reduction in the total nitrogen could not alone be expected to account for the increased stability of the juice; some significance was also

attributed to the reduction in total phosphate, calcium, and magnesium of the treated juice as revealed by spectrographic analysis.

In view of the importance of delaying the onset of fermentation in pasteurized apple juice after the bottles have been opened and the contents partially consumed, Challinor, Kieser, and Pollard's results might be anticipated to be of significant value in providing a solution to this problem. Their paper provides no data, however, concerning the quantities of resin required to prepare juices of high stability and also fails to comment on the organoleptic properties of the deionized juices. These points require careful investigation before consideration can be given to utilizing demineralization for the commercial production of apple juice of high fermentation resistance. Accordingly, we have studied Challinor, Kieser, and Pollard's technique with the above objects in mind and present the results of our own investigation in this paper.

METHODS

It was not our intention at this stage to investigate the relative importance of the trace elements presents in apple juice that are essential for the growth of microorganisms. Our attention was confined to: 1, examining whether a simple control test could be devised for indicating when demineralization had been carried out to an extent sufficient to confer stability upon the juice; 2, investigating whether cation removal alone would suffice without the subsequent necessity of removal of anions; and 3, observing the effect of demineralization upon the flavor of the juice.

Charley *(4)* has shown that fermentation in cider can be checked if the fermenting juice is centrifuged after the specific gravity has fallen several degrees and there is a pronounced decrease in the nitrogen content of the centrifuged juice. Challinor and Burroughs *(2)* demonstrated that fermentation can only be checked by this method after utilization of the readily assimilable nitrogen by the yeast is complete, although this did not enttirely explain the reason for retardation of fermentation.

Much of the nitrogen in apple juice is undoubtedly present in the form of basic amino nitrogen which should be capable of removal by resins of the cation-exchange type possessing a strongly acidic nature such as Zeo-karb 215. Engliss and Fiess *(5)* showed that all amino acids are taken up through the amino group when their solutions are passed through a resin of this type. On the other hand, anion-exchange materials only absorb the acidic amino acids, neutral and basic amino acids passing through unchanged.

Examination of Challinor, Kieser, and Pollard's results indicates that the cationic and anionic constituents were removed from the juice to a far greater extent than the total nitrogen. It appeared, therefore, that estimation of total nitrogen content by a micro-Kjeldahl method would prove a reliable guide to the efficiency of demineralization by ion-exchange resins. Accordingly, throughout our investigations we have used total nitrogen content as a control method for checking the efficiency of demineralization. We then wished to determine whether 3-stage demineralization as used by Challinor, Kieser, and Pollard, could be replaced by a single stage treatment through a cation exchange bed. Omission of the anion-exchange treatment, however, would involve retention of $—PO_4$, an essential yeast nutrient in the juice, and it was necessary for us to determine the extent to which its presence in the absence of cations might impair the stability of the juice.

Demineralization. For small-scale experimental work, columns of ion-exchange resins were not suitable for the experiments we had in mind. After regeneration, the columns must be maintained full of water which is eventually displaced by the liquid to be treated. Quantitative recoveries of juice by volume at original concentration are not then possible owing to dilution effects. On the other hand, if the water is removed from the bed by suction before admittance of the juice, flow of the latter through the column is impeded due to the presence of air and contact between juice and resin is incomplete.

In our preliminary experiments we sought to overcome these difficulties by agitating the resin with the juice in a vessel of large diameter, a method used by Gore *(6)* for reducing the acid content of citrus juices. We found, however, that the demineralized juice became cloudy due to the presence of very finely-divided resin. Eventually it was necessary to design an apparatus for treatment of small quantities of juice that would eliminate the above objections.

Demineralization apparatus. As shown in Figure 1, a disc of 120-mesh stainless steel wire gauze secured to a similar disc of stronger coarse mesh stainless steel gauze supports the resin bed inside a Cona funnel of 12-cm. diameter and 300-ml. capacity *(E)*. The extension tube of the funnel is cut off short and closed with a rubber bung carrying a glass tube of 5 mm. bore which is connected to the side arm of a 250-ml. inverted Buchner flask. The mouth of the latter is closed with a rubber bung carrying a tube *(C)* connected to a water tap, a tube *(B)* leading to a vacuum pump, and a delivery tube *(A)* running to a receiving vessel for collection of the demineralized juice.

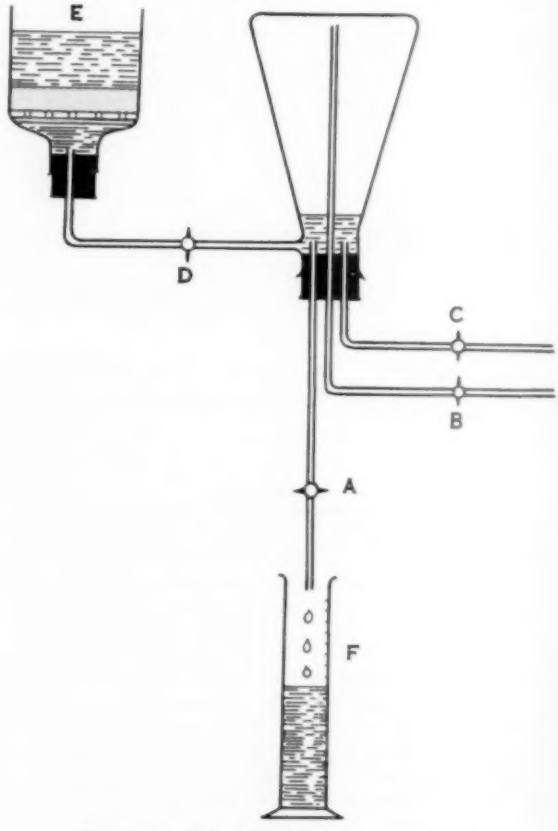

Figure 1. Demineralization apparatus.

A known weight of washed ion-exchange resin is placed in the ion-exchange vessel *(E)*, and stopcocks *D* and *B* are fully opened, after closing *A* and *C*. When all excess water has been removed from the bed by suction, stopcocks *D* and *B* are closed, *A* is opened and the water collected in receiver *F*. This receiver is then changed, a known volume of apple juice is poured carefully into vessel *E* taking care to avoid excessive disturbance of the resin bed, and the juice allowed to percolate through the bed and collected in receiver *F*, the rate of flow being controlled by regulation of stopcock *D*. Finally, *A* is closed, *D* and *B* are fully opened and the residual juice removed from the bed by suction, *A* finally being opened after closing *B* so that this juice also may be collected in receiver *F*. Dilution and loss of juice are held to a minimum obtained by this method. The resin bed is then back-flushed by admittance of a stream of water through *C* and *D*. Regenerating fluid is then added direct to vessel *E* and the bed finally washed and sucked dry as before.

RESULTS

The apple juice used in these experiments was a blend of several culinary and cider varieties. The ion-exchange materials used were Zeo-karb 215 for removal of cations, and De-Acidite E for anion removal. The minimum quantity of each resin required to reduce effectively the nitrogen content of apple juice was ascertained. For this purpose, 1,000-ml. batches of clarified, filtered apple juice were run through various quantities of each resin using the apparatus illustrated in Figure 1. The rate of percolation through the bed was approximately 33 ml. per minute. The demineralized juices were filtered and analyzed (Table 1).

TABLE 1
Proportions of Zeo-karb 215 and De-Acidite E required for demineralization of apple juice

Dry resin g. per liter juice	pH	Nitrogen	Specific gravity	Total sugars	Dry resin used, percent of weight of apple juice
		mg. per 100 ml.		*g. per 100 ml.*	
Zeo-karb 215					
0	3.17	9.83	1.050	10.33	0
25	2.71	7.06	1.045	8.91	2.5
50	2.48	3.96	1.045	8.43	5.0
100	2.26	2.19	1.041	8.45	10.0
150	2.40	1.25	1.039	8.04	15.0
De-Acidite E					
0	3.32	11.55	1.050	10.24	0
100	3.66	8.61	1.045	9.09	10.0
200	3.73	8.89	1.046	9.38	20.0
300	3.90	9.16	1.045	9.09	30.0
400	4.21	9.19	1.042	8.49	40.0

Increasing the proportion of Zeo-karb 215 to juice produced a corresponding increase in removal of total nitrogen. At least 150 g. per liter of this resin were required to reduce the nitrogen content to a low level. Only a small proportion of nitrogen was removed by De-Acidite E; weight of resin in excess of 200 g. per liter of juice produced no further decrease in nitrogen.

Stability of de-ionized juices. Batches of apple juice with nitrogen contents reduced to varying levels by treatment with different proportions of Zeo-karb 215, apple juice with anions removed by De-Acidite E, and untreated apple juice, were prepared for fermentation tests.

Choice of a suitable method for investigating juice stability, however, presented several problems. We wished to simulate as closely as possible the actual conditions to which the juices would be subjected when received by the consumer. Removal of the bottle closure would expose the juice to chance infection and after part of the contents had been consumed, the seal would be replaced. We decided, therefore, after pasteurization of all of the juice to inoculate them with a known amount of vigorous yeast culture, and then fill into clean sterile bottles to different filling heights, namely, full, ⅔-full, and ⅓-full. They were then closed with crown corks. Owing to the limited quantities of demineralized juices available, bottles of 7 fluid-ounce capacity were used for the tests.

The yeast culture was prepared from a vigorous mixed culture obtained by incubating slices of fresh apple peel in apple juice. The organisms were removed by centrifuging, well washed, and then suspended in sterile water. Each juice received an inoculation of approximately 500 yeast cells per ml. The bottled juices were stored in the laboratory at room temperature for several weeks and at intervals examined for alcohol content, sugar content, yeast count, and gas pressure. Results of the test are given in Table 2. All of the juices that received Zeo-karb treatment were drawn from the same storage tank as the untreated juice. The juice used for anion removal by De-Acidite E was drawn from a different tank possessiong original sugar and nitrogen contents of 10.24 g. per 100 ml. and 11.55 mg. per 100 ml., respectively.

Nitrogen content proved to be a valuable criterion of the efficiency of demineralization for improving the stability of the juices. The untreated juice with a nitrogen content of 10.73 mg. per 100 ml. was fermenting vigorously within 7 days, whereas the juice with a nitrogen content reduced to 0.86 mg. per 100 ml. had developed only slight fermentation in 81 days and the gas pressure in the bottle was too small to measure. Treatment with De-Acidite E had a stabilizing effect on the juice but to a much less extent than that of Zeo-karb 215.

There was a tendency for yeast multiplication to increase with the head-space in the bottle as substantiated by yeast counts, alcohol production, and decrease in sugar. Yeast multiplication was only accompanied by fermentation in the juices of higher nitrogen content. In the de-cationized juices, the yeast cells showed distortion with shrunken cell contents and irregular cell membranes, accompanied by degeneration and autolysis. Gas pressures provided good indication of the progress of fermentation in each series of juices and in the bottles with smaller head-space the fermentation was retarded when the pressure became high.

These results demonstrate that if a high proportion of resin is used, a single treatment with Zeo-karb 215 will impart a high degree of stability to apple juice without the necessity of anion removal.

Effect of demineralization on flavor. We found that all of our samples of apple juice had lost most of their flavor and aroma after demineralization. White *(9)* has shown for one sample of apple juice that the volatile flavor fraction contained approximately 92% alcohols, 6% carbonyl compounds, and 2% esters, and consti-

TABLE 2

Relation between nitrogen content and rate of fermentation of apple juice

	Untreated juice				Zeo-karb 215-treated				Zeo-karb 215-treated				De-Acidite E-treated			
	10.73 mg. N per 100 ml.				3.05 mg. N per 100 ml.				0.86 mg. N per 100 ml.				8.80 mg. N per 100 ml.			
	Days	Full	⅔	⅓	Days	Full	⅔	⅓	Days	Full	⅔	⅓	Days	Full	⅔	⅓
Total sugars, g. per 100 ml.		9.98	10.06	10.06		9.98	9.98	9.98		8.94	8.94	8.94		7.87	7.87	7.85
Alcohol, % v./v.	5	0.4	0	0	5	0	0	0	5	0	0	0	0	0	0	0
Yeasts per ml., × 10^5		15.0	0.005	0.005		0.005	0.005	0.005		0.005	0.005	0.005		0.005	0.005	0.005
CO_2 p.s.i.g.		2	2	0		0	0	0		0	0	0		0	0	0
Total sugars, g. per 100 ml.		9.98	8.98	8.62		8.89	8.04	8.79		8.88	8.01	8.21		7.29	7.16	7.08
Alcohol, % v./v.	7	0.4	0.66	0.93	47	0.26	0.69	0.69	47	0	0.26	0.26	21	0	0	0.13
Yeasts per ml., × 10^5		50	100	Max.[b]		30	35	100		45	35	35		Max.	Max.	Max.
CO_2 p.s.i.g.		20	15	13		+[a]	+	+		+	+	+		—	4	2.5
Total sugars, g. per 100 ml.		9.90	7.93	7.16		8.45	8.04	8.61		8.67	8.01	8.21		6.63	5.96	5.96
Alcohol, % v./v.	10	0.53	1.00	1.34	66	0.66	0.69	0.80	66	0	0.26	0.26	36	0.80	0.83	1.20
Yeasts per ml., × 10^5		Max.	Max.	Max.		35	100	Max.		50	35	35		Max.	Max.	Max.
CO_2 p.s.i.g.		38	51	30		+	7	5		+	+	+		32.5	25	20
Total sugars, g. per 100 ml.		9.42	6.97	5.68		7.78	7.23	7.87		8.21	8.06	8.06		4.32	3.64	3.57
Alcohol, % v./v.	13	0.53	1.00	1.34	81	0.83	0.83	0.98	81	0.13	0.26	0.26	60	2.40	2.38	2.54
Yeasts per ml., × 10^5	—	—	—	—		35	Max.	Max.		35	35	35		Max.	Max.	Max.
CO_2 p.s.i.g.		55	80	43		5	7.5	5		+	+	+		120	70	45

[a] The + sign represents a pressure too small to measure.

[b] Max. represents a yeast count higher than the maximum capable of estimation by the method used.

tutes a total concentration in the original juice of approximately 50 p.p.m. Presumably these components are removed by adsorption when apple juice is demineralized, for we found it possible to recover a little of the flavor by elutriation of the resin bed with distilled water. The amount recovered was, however, insufficiently intense when returned to the juice to render the latter palatable even after reduction of the acidity to its original value.

Loss of flavor from juice demineralized by Zeo-karb 215 is shown in Table 3. For the purpose of this experiment, juice was demineralized through beds of Zeo-karb 215 of varying depth in columns 3 cm. in diameter. Dilution effects were minimized and adequate contact between resin and juice was ensured by filling the columns with a portion of the juice before addition of the resin. The proportion of resin to juice was 150 g. (on dry weight) resin to each liter of juice treated. The pH, specific gravity, total reducing sugar, volatile flavor fraction [determined as ester content by the method of Challinor and Burroughs *(1)*], and nitrogen content of each batch of demineralized juice were estimated and compared with the original analyses of the untreated juice. Increased column depth resulted in increased overall efficiency of the resin bed. This had an adverse effect on the flavor, for both volatile flavor fraction (ester content) and sugar were reduced in quantity, and there was a pronounced increase in acidity as reflected in the change in pH value. Over 25% of the original sugar and approximately 85% of the esters were removed in the 64.0 cm. column and together with the increase in acidity, this constituted a very serious impairment of flavor which was confirmed by organoleptic tests.

Retention of flavor in demineralized juice. The value of demineralization for rendering apple juice stable against fermentation is negated unless a method can be found for retaining the flavor. This difficulty can be overcome by removing the volatile flavor by "flashing" the juice before treatment with Zeo-karb 215. Milleville and Eskew *(7)* have described a process for recovering the volatile odorous fraction of apple juice in a concentrated form. For this purpose we have developed a small-scale laboratory apparatus for production of a flavor concentrate from small quantities of juice.

Laboratory apparatus for flavor recovery. The apparatus illustrated in Figure 2 is based upon the apparatus designed by Mottern and von Loesecke *(8)* for the deaeration of fruit juices. The "flashing" chamber *A* consists of an evacuated inverted 3-liter flask into which the juice is drawn by suction from reservoir *C* after passage through a 600-cm. glass coil with an internal diameter of 3 mm. which is heated by a water jacket maintained at a temperature of 194° F. (90° C.). The juice is atomized in the flashing chamber through a glass jet *M* with an orifice diameter of 1 mm. and the volatile fraction together with some water vapor leaving the "flashing" chamber through *D* is condensed in a coil *E* which is surrounded by brine circulating at 28.4° F. (−2° C.) and collected in a flavor receiver *F* which is also held under vacuum. The stripped juice is collected in a receiver *G* after passage through a cooler *L* having an effective length of 360 cm. and an internal diameter of 4 mm. Build up of pressure in *G* is prevented by connecting the side arm of the latter to the flashing chamber *A* by a vapor tube *H*.

A pressure of 2.5 mm. Hg. was maintained in the apparatus throughout operations by means of a vacuum pump. It was found unnecessary to continue operation of the pump after the required degree of vacuum had been attained, thus reducing loss of uncondensed flavor from the flavor receiver *F*.

The temperature of the juice was maintained at 176-185° F. (80-85° C.) as shown by a thermometer *I*, by controlling the flow of juice through stopcock *K*.

TABLE 3

Influence of depth of resin-bed on removal of esters and sugars from apple juice

Volume of juice	Dry resin	Depth of bed	pH	Specific gravity	Total sugars	Esters	Nitrogen
ml.	*g.*	*cm.*			*%*	*mg. per 100 ml.*	*mg. per 100 ml.*
Untreated	0	0	3.03	1046	12.26	21.04	10.01
500	75	20.0	2.10	1038	9.22	5.88	1.80
800	120	31.5	2.06	1038	9.38	5.88	0.80
1100	165	47.5	1.92	1038	8.98	4.61	0.77
1600	240	64.0	1.80	1036	8.56	3.25	0.93

With this apparatus, 2400 ml. of flavor concentrate were obtained from 19,440 ml. of juice. The flavor concentrate alone was then re-circulated through the apparatus to give a final yield of 250 ml. The stripped juice was restored to its original volume of 19,440 ml. by addition of distilled water. The concentration of the flavor fraction was assessed by organoleptic tests to be concentrated approximately 50 times. Addition of 2 ml. of this concentrate to every 98 ml. of the stripped, fully diluted juice restored the apple flavor and aroma to its original intensity.

Stability test on reconstituted demineralized juice. The stripped juice was demineralized with 15% of its weight of Zeo-karb 215 (dry basis). The acidity was restored to its original value by partial neutralization, and sugar was also added to compensate for the loss in

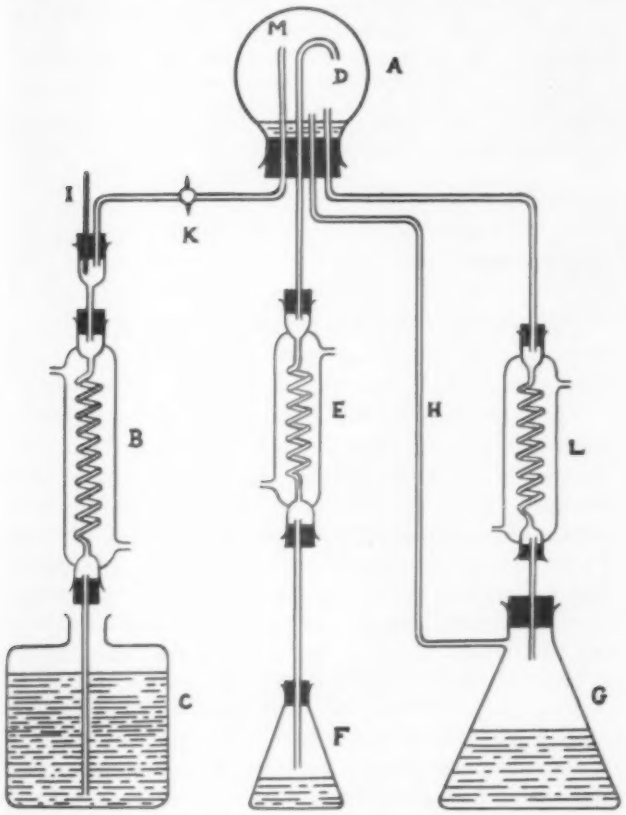

Figure 2. Flavor recovery apparatus.

the resin bed. Finally, 2 ml. of flavor concentrate was added to each 98 ml. of juice, which was then bottled and pasteurized. It remained perfectly bright, and flavor and character were judged to be excellent, although some of the body of the original juice was lacking.

A washed suspension of a vigorous yeast culture was added to a series of 7-ounce bottles filled to different heights with the reconstituted pasteurized juice and held under observation in the laboratory at ordinary temperature for a period of 74 days.

Frequent tests made during the first 70 days showed no evidence of fermentation, and although there was some increase in the yeast content, microscopical examination revealed that the cells were shrunken and depleted of essential nutrients. Throughout this period there was no impairment of flavor apart from a slight loss in sweetness.

In the bottles which were only partially full, fermentation developed 4 days later with production of alcohol and carbon dioxide. The carbon dioxide in the $\frac{2}{3}$-full bottles was insufficient to register on the pressure gauge employed. There was a heavy yeast sediment in all the bottles, but lack of oxygen in the full bottles would acount for the absence of alcoholic fermentation. Loss of sugar in these samples was no doubt due to utilization by the yeast for its survival under conditions of restricted oxygen supply.

TABLE 4

Stability of reconstituted deionized apple juice of pH 2.2 and containing 1.51 mg. nitrogen per 100 ml.

	Analyzed before inoculation	Inoculated, analyzed after 74 days		
		Full	⅔-full	⅓-full
Specific gravity	1.050	1.042	1.041	1.040
Sugar, g. per 100 ml.	10.2	8.37	8.73	8.37
Alcohol, % v./v.	0	0	2.5	3.5
Yeast per ml. × 10^6	0.005	5.0	1.5	3.0
CO_2 p.s.i.g.	0	0	0	2.5

DISCUSSION

We have shown that it is possible to produce a full-flavored apple juice possessing high resistance towards fermentation. The single-stage demineralization treatment advocated by us would involve a much lower capital outlay than that required for a 3-stage installation including 1 column of anion-exchange resin. The total nitrogen content of the treated juice can provide a useful criterion to the efficiency of demineralization, but it is felt that further research will be required before adoption of the process for commercial usage can be contemplated. Some varieties of juice may require treatment with a higher proportion of resin than that employed in our experiments depending on the concentrations of cations of those trace elements which are essential yeast nutrients. Special precautions must be taken so that the demineralized juice will not acquire additional trace elements after treatment. Adequate cleansing of juice receptacles and containers is essential if contamination is to be avoided.

In addition to providing a means for delaying onset of fermentation in apple juice of natural strength, the process also offers possibilities for the stabilization of apple juice syrups for dilution and carbonation. It may be possible to reduce or even omit the addition of preservative in drinks of this nature. It is considered that further investigations may establish an important role for demineralization in apple juice processing.

SUMMARY

The method suggested by Challinor, Kieser, and Pollard for increasing the resistance of apple juice to fermentation by treatment with ion-exchange resins has been investigated. It has been found that cation removal alone imparts a high degree of stability to the juice, and that determination of the residual total nitrogen content is a good indication of the efficiency of the treatment.

Demineralization involves some loss of sugar and also seriously impairs the flavor. A high proportion of the natural flavor appears to be removed by adsorption on the resin bed and cannot be recovered. Loss of flavor may be avoided if the volatile flavor fraction of the juice is removed by "flash heating" and concentration before submitting the juice to demineralization. The flavor fraction may subsequently be returned to the demineralized juice, together with additional sugar after restoration of the acidity to its original value to yield a full-flavored juice of attractive character with high resistance to fermentation.

Acknowledgment

The authors wish to express their thanks to the directors of Seager, Evans and Co., for granting permission to publish the results of this investigation, and also to Mr. J. Knight for assistance with the analytical work. The samples of Zeo-karb 215 and De-Acidite E used in the demineralization experiments were obtained from the Permutit Co., of London, England.

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Book Review

Statistical Methods for Chemists. By W. J. Youden. 126 pages, John Wiley and Sons, New York, N. Y. 1951. Price $3.00.

Attractively bound and well printed on good paper, this book fulfills the promise of its title. The ten chapter headings are: Precision and Accuracy, Measurement of Precision, Comparison of Averages, Resolution of Errors, Statistics of the Straight Line, Analysis of Variance, Interaction between Factors, Requirements for Data, Arrangements for Improving Precision, Experiments with Several Factors. An appendix contains a useful set of mathematical tables. The index is small, poorly prepared, and might well have been omitted or combined with the table of contents.

This is no textbook on statistics, but a thoughtful treatise for the mature chemist who wishes to express his experimental findings in statistical language. The chemist will find no mention of such topics as large number theory, and no derivation of equations of interest only to the professional statistician. Instead, the author has addressed himself to the specific audience of those who make measurements and interpret data. In the twenty practical examples, the reader is shown how experiments can be planned, and results expressed statistically. The food chemist might wish that at least one of these examples dealt with biochemistry or food technology. Many chemists may feel with Dr. David M. Bonner, the current recipient of the Eli Lilly Award, "If you design your experiments properly, you don't need a statistical analysis to prove your results." Dr. Youden meets this criticism squarely by devoting four of his ten chapters to the design of experiments. The whole book is imbued with the idea of previous planning to anticipate possible interactions between experimental factors.

G. L. Mack

Table Wines. By M. A. Amerine and M. A. Joslyn. 397 pages, 38 tables and 33 figures. University of California Press, Berkeley, California, 1951. Price $4.50.

This volume is the successor to two previous publications of the University of California: Agricultural Extension Service Circular 88," Elements of Wine Making," by M. A. Joslyn and W. V. Cruess, and Agricultural Experiment Station Bulletin 639," Commercial Production of Table Wines," by M. A. Amerine and M. A. Joslyn. While material from these publications has been freely drawn upon, much new information has been added. The book has little resemblance, both in appearance and arrangement, to its precedents.

The book is based on investigations of table wines since the repeal of prohibition in the University of California divisions of food technology and viticulture; on the authors' observations of the wine industry; and on their review of the literature.

Four major parts are presented in the book:

1. The design and construction of the winery, its equipment, and sanitation problems are thoroughly discussed in the first part. A schematic floor plan of a winery handling about 1,000 tons of grapes a year with a storage capacity of 200,000 gallons is presented. Several large pictures showing the winery construction and equipment are included in this part.

2. The second part of hte book recommends the proper procedures for making various types of table wines from harvesting the grapes to finishing the new wines. Detailed procedures for making dry red wines, dry pink wines, dry white wines, sweet table wines, sparkling and carbonated wines are carefully described.

3. The third part of the book is devoted to the preparation of wines for market. It consists of finishing, analyzing, discussions of diseased wines, and the timely subject of winery by-product. Much of the materials presented in this part are the original works of the authors and their associates.

4. The last part of the book is more or less "academic." It consists of the technological principles of producing table wines.

and the theories involved in alcoholic fermentation. The composition of grapes and table wines is thoroughly exploded, with several valuable tables presented.

The Appendixes contain several basic and reference tables which are needed regularly by wine makers. One appendix lists the general literature references and citations. Domestic and foreign books and bulletins concerning table wines are included in this part.

Winemakers and students of enology will want copies of this book on their own book-shelves. Although the volume is devoted principally to table wines, much of the information included is applicable to other types of wines. It can be also used as a general reference in enology.

Comments on this book are not complete unless credits are given to its designer, Mr. A. R. Tommasini. The general appearance of the book represents the "new look" of scientific and technological books. Several interesting woodcuts appear in the appropriate places of the book. A short summary of the subject matter is printed under the title of each chapter as well as each sub-title in the chapter. It gives the reader a general idea of what he is about to read.

Most of the photographs run all the way to the edges of the page utilizing the entire margin space. It makes the pictures look larger and clearer. These and other typographical innovations appear throughout the book and its cover represents the ingenuity of the designer.

E. H. Wiegand

Food Industries Manual. T. Crosbie-Walsh, Editor. 1036 pages. Leonard Hill, Ltd., London, 17th ed., 1951. Price 42 shillings, about $6.50.

This manual deals with the manufacture, preserving, packing, and storage of food products. Information is presented in encyclopedic form under sections on milling, sugar confectionery, canning and preserving, meat products, pickles and sauces, the dairy industry, food dehydration, edible fats and fatty foods, fruit juice products, food storage and refrigeration, food handling equipment, packaging, the boiler house, and composition of foods.

The topics treated in each section are indicated by words set in bold-faced type and arranged alphabetically for ready reference. Cross references are given under many of the topics to discussions on related subjects in other sections of the manual. An 11-page subject index also aids in finding information readily.

The principles of different processes used in the production of food products are stressed, and basic formulas are given as useful starting points for experimentation in the commercial production of breads, cakes, confectionery, pickles, and other food products.

Obsolete matter has been deleted and information added regarding new processes and raw materials. There is no mention, however, of some of the food products now so popular in the United States, such as prepared cake and biscuit mixes, canned baby foods, soluble coffee and tea powders, and frozen precooked foods.

At the end of the manual is a 21-page list of names and addresses of industrial concerns, manufacturers of various products, and trade names and marks, all representative of British food industries.

In the discussions of the different processes relating to food preservation, recognition is given to the work of investigators in the United States as well as in Great Britain. Any one concerned with food manufacture and preservation, irrespective of nationality, will find much basic information in the manual.

B. E. Proctor.

Food and Nutrition. By E. W. H. Cruickshank, 443 pages. Williams and Wilkins Co., Baltimore. Second Edition, 1951. Price $6.50.

This outstanding British text deserves careful reading by all nutritionists concerned either with feeding families or whole nations of people. The first quarter of the book presents the practical problems of feeding the world's people and feeding the British nation during the past decade. The second quarter of the work covers the conventional science of nutrition including clearly written and modern chapters dealing with metabolism, energy requirements, proteins, minerals and vitamins. In this section the historical portions are hackneyed and threadbare from repetition by other texts. British names for vitamins such as "axerophthol" for vitamin A are employed. Part of the illustrations are as shop worn as the history but part are newer and refreshing. However this section can be read with profit by even the senior citizens among nutrition specialists since it brings many loose ends together. The latter half of the text contains excellent chapters upon foods such as milk, bread, protein rich foods, vegetables, fruits, nuts and oils. This latter half also contains chapters upon preservation of foods, dental caries, dietary standards and appraisal of nutritional status. Well selected lists of references follow each chapter. The index is adequate.

For Americans this text is worth-while both because of its substantial review of basic modern knowledge and because it stresses British science and experience without unduly neglecting American contributions. One major asset of the work is that it omits the usual mass of organic formulas for amino acids, vitamins and other organic compounds important in nutrition. Even the timid and uninitiated will not be rendered allergic to nutrition by this work which omits the usual hieroglyphics of organic chemistry. The overall point of view of this text is that of the modern nutritionist who realizes that he or she must not only be well grounded in biochemistry, physiology, pathology and biology but that a basic knowledge of foods, food processing and food economics is equally essential. For this last reason this work will prove especially useful in the private libraries of physicians, public health nurses and social workers.

A few minor errors in the work such as that Chittenden worked in Boston, or that rhubarb and spinach should be condemned because of their oxalate contents, can be overlooked either because the author is not American or not familiar with some of the later knowledge such as that concerned with oxalates.

Clive M. McCay.

Notes and Letters

Electronic Egg Grading

Electronic egg grading originated in 1937 in my laboratory at Cornell University as an incidental outcome of experimental work on the detection of fertility in intact fresh eggs *(2, 3)*. Observations revealed a high variability in the radio-frequency conductivity of individual eggs and a consistent increase in their conductivity as they aged. It became apparent that the electronic method, if mechanically improved, could be a useful tool for evaluating the internal quality of eggs *(1, 4)*.

The chief requirement was to provide automatic compensation for variations in the size of the egg, since power absorption in the egg coil is in direct relationship to the mass of the egg. In the latest models of the instrument, this handicap has been overcome, as shown by the accompanying scatter diagram of conductivity readings on fresh eggs of various weights (Figure 1).

Figure 1. Scatter in conductivity readings (at frequency of 65 megacycles) of 481 fresh intact eggs of various weights. It was observed (1, 4) that eggs of highest readings are more perishable in storage than eggs of lowest readings.

The instruments are so sensitive, however, that any slight disturbance of the egg coil or other parts of the circuit (as by inadvertent jarring, etc.) may produce relationship changes which displace the reference point and/or cause the weight and shape of the egg to influence conductivity values very markedly. Therefore, it is still possible to obtain inconclusive results, if the delicacy of the instrument is not appreciated.

Changes in the conductivity readings of stored or aging eggs are consistent with simultaneous changes in other values, such as the opened-egg score and albumen index (Figure 2), as well as weight loss and increase in air-cell size. In deteriorating eggs, the correlation between conductivity and the condition of the albumen

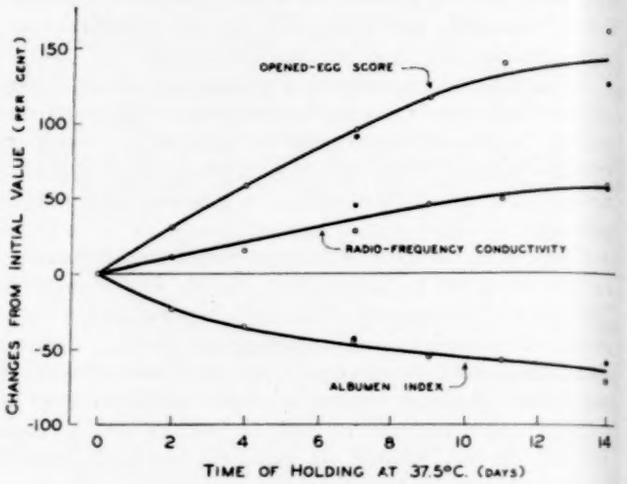

Figure 2. Relationship of conductivity readings to the opened-egg score and albumen index in intact eggs held at 37.5° C. Solid dots represent Cornell University unpublished data, and circles represent data from the U. S. Bureau of Animal Industry, Beltsville, Md. (personal communication).

appears to be especially close. The trend in the conductivity readings of aging eggs is of such magnitude that it is apparent even if the instrument is slightly out of adjustment.

The electronic method of egg grading represents an attempt to make a practical application of a physical principle. The soundness of all aspects of the method has not yet been completely established; on the other hand, there is considerable evidence to indicate a correlation between the egg's conductivity and its internal quality and future perishability *(1, 4)*. Comparisons of the results of the electronic evaluation of eggs with results obtained by currently used methods are subject to various interpretations, especially since some of the standard procedures have definite limitations *(5)*.

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April 24, 1952.

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Biochemical Prevention of Flavor and Chemical Changes in Foods and Tissues Sterilized by Ionizing Radiations[a, b]

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Department of Food Technology, Massachusetts Institute of Technology, Cambridge, Mass.

(Manuscript received April 29, 1952)

When ionizing radiations are used to sterilize foods and tissues, side-effects are produced that cause changes in their flavor and color and sometimes their texture. Irradiation of these materials in inert atmospheres or in vacuo and especially in the frozen state minimizes but does not prevent such changes.

A biochemical approach having potentialities for prevention of off-flavor and other undesirable chemical changes in foods and tissues is described. This approach is based on a theoretical analysis of the radiochemical phenomena occurring in the reaction of ionizing radiations with water and on experimental data obtained in cathode-ray studies with certain compounds in aqueous solutions.

The undesirable effects resulting from radiation sterilization are ascribable to free radicals produced in the reaction of ionizing radiations with water. These effects can be reduced or prevented by use of free-radical acceptors, which protect the solute (flavor molecule or nutrient) by competing for the free radicals. This principle has been applied successfully also to prevent hemolysis in suspensions of red blood cells when they are irradiated.

In the years from 1946 to 1952, many investigations have been conducted on the sterilization of foods, tissues, and pharmaceuticals by ionizing radiations *(12)* produced by Van de Graaff accelerators. During this period the following facts have been observed:

a. All types of microorganisms can be destroyed by sufficient doses of ionizing radiations in any conceivable type of container.

b. The amount of radiation dose required is dependent on the type and the number of the spoilage organisms present, as well as on the type of food material to be irradiated.

c. In this treatment, side-effects such as changes in flavor, texture, and color may be produced. The extent of each of these changes is dependent on the particular food, drug, or tissue being irradiated and on the dose used. In general, side effects are produced by much lower doses than those required for sterility.

d. These side effects can be minimized but not necessarily entirely avoided by irradiation of the material in the frozen state.

e. Irradiation in inert atmospheres or in vacuo has also been shown to be helpful, but under such conditions a higher radiation dose may be required for a given degree of bacterial inactivation or sterility than is required in normal atmospheres.

With the above facts in mind, a biochemical solution to the prevention of the undesirable side effects produced in irradiated materials was postulated, and the potentialities of this approach in cathode ray studies with certain compounds in aqueous solutions have been investigated. A preliminary report of some phases of this investigation has been published by Proctor and Goldblith *(13)*. This biochemical approach is based on an analysis of the radiochemical phenomena occurring in the reaction of ionizing radiations in aqueous (or aquoorganic) solutions. These phenomena may be summarized as follows:

On passing through water, ionizing radiations lose energy, in part at least, by causing water molecules to ionize:

$$H_2O \sim\sim\sim\sim\sim\sim\longrightarrow H_2O^+ + e^- \qquad (1)$$

The ejected electron gets away from the positive water ion and eventually becomes attached to another molecule, forming a negative water ion:

$$e^- + H_2O \longrightarrow H_2O^- \qquad (2)$$

Alternatively, the ejected electron may combine (but probably rarely does so) with a hydrogen ion to form a free hydrogen atom:

$$e^- + H^+ \longrightarrow H \qquad (3)$$

The positive water ion interacts with its neighboring water molecules and in the process leads to dissociation:

$$H_2O^+ \longrightarrow H^+ + OH^\cdot \qquad (4)$$

The resulting negative water ion from Equation 2 dissociates:

$$H_2O^- \longrightarrow OH^- + H \qquad (5)$$

In addition to the reaction illustrated in Equation 4, a hydroxyl radical may be formed by ejection of electrons from hydroxyl ions normally present in water:

$$OH^- \longrightarrow OH^\cdot + e^- \qquad (6)$$

The electrons so produced may yield hydrogen atoms, according to Equation 3.

The important products formed in the reaction of ionizing radiations with water are the $OH^\cdot$ and the H atom. The former is a strong oxidizing agent and the latter a powerful reducing agent. Any oxidizable solutes that are present will, therefore, be oxidized by these radiations through the $OH^\cdot$ produced and any reducible solutes will be reduced through the H atoms produced.

The H atom and the $OH^\cdot$ may recombine if they are close enough together (Equation 7), and in this case there will be no net change:

$$H + OH^\cdot \longrightarrow H_2O \qquad (7)$$

However, on a molecular scale the distance between the hydrogen atom and the hydroxyl radical is so great that other things are likely to happen (such as the oxidation of an oxidizable solute or the reduction of a reducible solute) before diffusion brings them together.

[a] Contribution No. 124 from the Department of Food Technology, Massachusetts Institute of Technology.

[b] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Michigan, June 8-12, 1952.

In food materials, which are extremely complex substances, a number of components are present that could react with free radicals. Among these are the components of flavor which, when irradiated, produce an off-flavor that is characterized by some members of taste panels as "oxidized." What conceivably happens is explained schematically in Equation 8:

$$\underset{\text{molecule}}{\text{Flavor}} + OH^{\cdot} \longrightarrow \underset{\text{flavor}}{\text{Oxidized}} + OH^{-} \qquad (8)$$

With enzymes, the type of reaction that may take place is illustrated in Equation 9. In this instance, an enzyme has been chosen for illustration that depends on a sulfhydryl group for its activity.

$$\underset{\text{(reduced)}}{2\ \text{Enzyme-SH}} + OH^{\cdot} \longrightarrow \underset{\text{(oxidized)}}{\text{Enzyme-S-S-Enzyme}} + OH^{-} \qquad (9)$$

The above has been amply demonstrated by Barrón *(2)* in his study on phosphoglyceraldehyde dehydrogenase irradiated by X-rays and reactivated by reduced glutathione (—SH).

The discussion above forms the rationale of a proposed biochemical approach for the prevention of off-flavors and other side reactions produced by ionizing radiations, based on the addition of a "free radical acceptor" (FRA) to the material that is to be irradiated. Forssberg *(6)* demonstrated that catalase could be protected from X-rays by the addition of a second component to the system, which absorbed some of the primary X-ray products. In later work, Forssberg *(7)* applied this principle to animal experiments with encouraging results.

EXPERIMENTAL

To demonstrate the protection afforded by free radical acceptors, quantitative kinetic studies were undertaken with pepsin (crystalline). The pepsin was irradiated in aqueous solutions (glass-redistilled water), in acetate buffer, and in acetate buffer plus sodium-d-isoascorbate and niacin, respectively. The activity of the pepsin was determined by the method of Anson *(1)*.

Irradiation studies were also made with aqueous solutions of histidine.HCl plus D-isoascorbic acid, with suspensions of red blood cells in vitro plus sodium sulfite or sodium D-isoascorbate, and with samples of chopped meat products (cured) plus ascorbic acid or one of its derivatives. Histidine was determined chemically by a colorimetric method *(3, 9)*.

The various liquid samples were irradiated at room temperature in thin-walled glass ampules of 5-ml. capacity. The chopped meat products were those of a commercial packer, containing the usual curing agents but repacked into oblong sardine cans after thorough mixing. The repacked meat products were then irradiated by crossfiring, that is, by exposing the cans from both sides, through the shortest dimension of the can. The source of ionizing radiations (cathode rays) was a 3 m.e.v. pressure-insulated Van de Graaff Accelerator *(14)*.

RESULTS AND DISCUSSION

The effects of ionizing radiations on the retention of pepsin in three different concentrations in aqueous solution and in acetate buffer are presented in Figures 1 and 2. The rate of destruction of a given dose of radiations was less, the more concentrated the solute.

From the slopes of the curves in Figures 1 and 2, the quantities of radiation to produce 63% destruction of the enzyme ($e^{-1} = 0.368$), that is, the inactivation doses (D_0) have been calculated *(5)* and are recorded in Table 1. Division of the inactivation dose by the

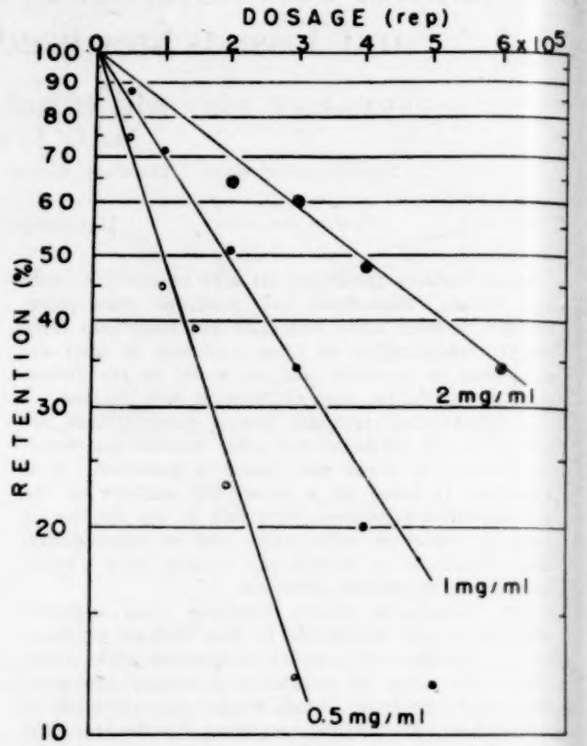

Figure 1. Effects of cathode rays on aqueous solutions of pepsin of different concentrations.

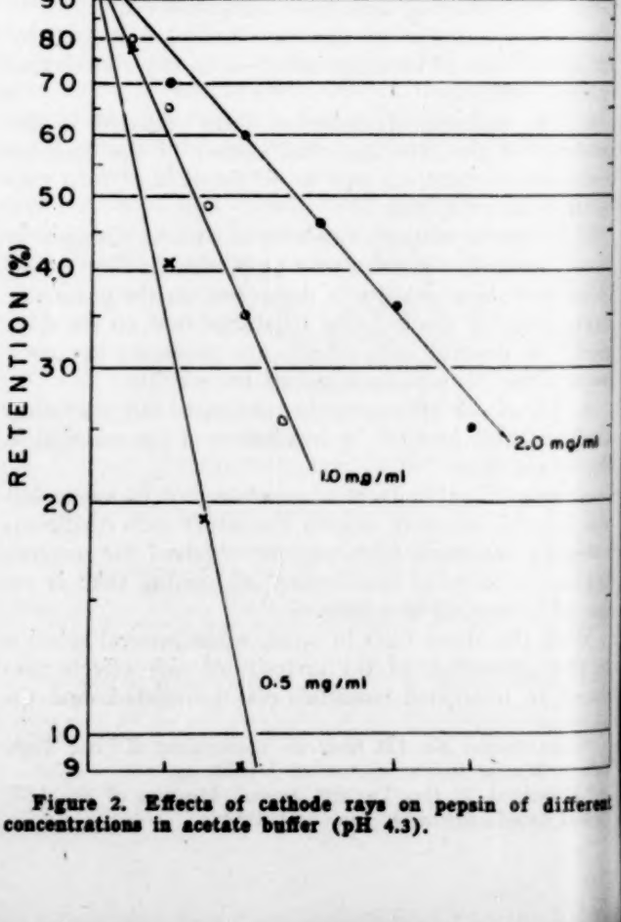

Figure 2. Effects of cathode rays on pepsin of different concentrations in acetate buffer (pH 4.3).

TABLE 1

Effect of cathode rays on pepsin in aqueous solution and in acetate buffer

Solvent and concentration (C) of solute (I)	Inactivation dose (D_0)	Specific inactivation dose (D_0/C)
mg. per ml.	*rep*	*rep. per g. per ml.*
Water	146,000	2.92×10^8
0.5	280,000	2.80×10^8
1.0	562,000	2.81×10^8
2.0		
		Average: 2.84×10^8
Acetate buffer[a]	185,000	3.70×10^8
0.5	395,000	3.95×10^8
1.0	765,000	3.83×10^8
2.0		
		Average: 3.83×10^8

[a] Concentration was 20.7 mg. per ml.; the pH 4.3 (pH at which pepsin is most stable).

concentration of the pepsin gives the specific inactivation dose (D_0/C).

The values for the specific inactivation dose indicate that the average amount of energy required to inactivate a given quantity of the pepsin in a given solvent was the same regardless of the concentration of the solute. This was true for pepsin both in aqueous solution and in acetate buffer. However, in the latter case more energy was required to inactivate a given quantity of the enzyme (3.83×10^8 rep as compared with 2.84×10^8 rep per g. per ml.).

This greater energy requirement for inactivation is probably explained by the protection afforded the pepsin by the acetate buffer. This protection may be expressed mathematically as follows *(4)*:

$$Q = \frac{D_{o(I+P)} - D_{o(I)}}{D_{o(I)}} \times \frac{C_I}{C_P}$$

where Q is the protective quotient; $D_{o(I+P)}$ is the inactivation dose for the indicator plus protector; $D_{o(I)}$ is the inactivation dose for the indicator alone; C_I is the concentration of the indicator that is present; and C_P is the concentration of the protector that is present.

According to the experimental data in Table 1, the average values for $D_{o(I+P)}$ and $D_{o(I)}$ are 3.83×10^8 and 2.84×10^8 rep per g. per ml. The concentration of the indicator (enzyme) was 1.0×10^{-3} g. per ml. and that of the protector (buffer) was 20.7×10^{-3} g. per ml. The protective quotient of buffer is then calculated from these values as follows:

$$Q = [(3.84 \times 10^8 - 2.84 \times 10^8)/(2.84 \times 10^8)] \times [(1.0 \times 10^{-3})/(20.7 \times 10^{-3})] =$$

$$Q = (0.99/2.84) \times (1.0/20.7) = 0.0168$$

In experiments in which the enzyme pepsin was used at a single concentration (1.0 mg. per ml.), the efficacy of protective agents (P) other than acetate buffer as free radical acceptors was evaluated. For example, with increasing quantities of sodium D-isoascorbate in the solution of pepsin and acetate buffer there was increased protection of the enzyme (Figure 3). Indeed, the pepsin was well protected by the sodium D-isoascorbate even in low concentrations.

A similar effect was observed in a study of the protective effect of niacin on pepsin in acetate buffer (Figure 4). In fact, niacin, which in itself is relatively

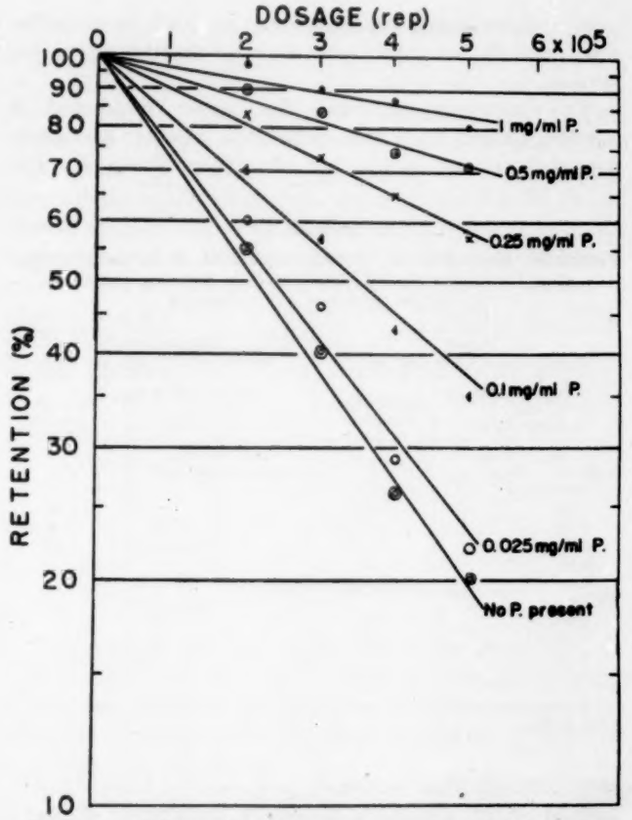

Figure 3. Effects of cathode rays on pepsin (1.0 mg. per ml.) in acetate buffer (pH 4.3) in the presence of different amounts of sodium D-isoascorbate (P is protector).

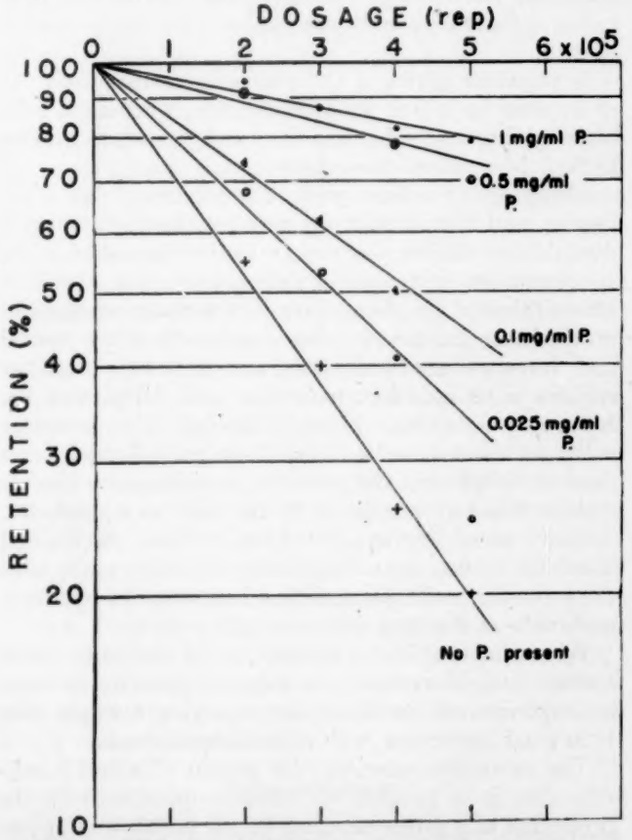

Figure 4. Effects of cathode rays on pepsin (1.0 mg. per ml.) in acetate buffer (pH 4.3) in the presence of different amounts of niacin (P is protector).

very radioresistant *(10, 11)*, was as good as or better than sodium D-isoascorbate as a protective agent for pepsin.

The protective quotients for pepsin irradiated in acetate buffer with the protective agents sodium-D-isoascorbate and niacin are shown in Table 2. The

TABLE 2

Protective quotients for pepsin irradiated in acetate buffer [4] and for Histidine.HCl irradiated in aqueous solution in the presence of protectors

Pepsin (concn. = 1.0 mg. per ml.)		Histidine.HCl (concn. = 1.0 mg. per ml.)	
Protector (P) and concentration	Protective quotient	Protector (P) and concentration	Protective quotient
mg. per ml.		*mg. per ml.*	
Sodium D-iso-ascorbate		D-isoascorbic acid	
0.025	4.60	0.1	3.06
0.10	6.40	0.5	1.26
0.25	8.20	1.0	1.72
0.50	7.67		
1.0	7.58		
Niacin			
0.025	19.68		
0.10	9.84		
0.50	9.00		
1.0	6.34		

[4] Concentration was 20.7 mg. per ml.; pH 4.3 (pH at which pepsin is most stable).

protective quotient has been proposed by Dale, Davies, and Meredith *(4)* as a means of comparing the protective power of different substances. Examination of the formula shows that Q, the protective quotient, expresses the ratio of the dose taken up by the protector to that taken up by the indicator for equal weights of each.

A protector giving a Q value greater than unity is of interest as a free radical acceptor, whereas a protector giving a Q value less than unity (such as acetate buffer) is of minor consequence.

Changing Q values were also observed by Dale, Davies, and Meredith. In general, such values imply a diminishing efficiency of the protective substance as its concentration is increased. Dale, Davies and Meredith have explained the phenomenon of the changing quotient on the basis that the protector reacts with a free radical and becomes activated, that the activated protector collides with indicator molecules and deactivates the indicator molecules. Hence, although the protector molecule has removed a radical from the solution, it can pass on the effect of the radical to the indicator molecule and in this way not fulfill its function as a protector. Another possibility, as pointed out by Dale, Davies, and Meredith, is that the solvent molecules may collide with the protector molecules and thus inactivate the protector molecules so that they can no longer protect.

By calculating the probabilities of collision, Dale, Davies, and Meredith have found it possible to draw an experimental curve for the changing quotient that is in good agreement with experimental results.

The protective quotients for pepsin (Table 2) indicate that it is possible to measure quantitatively the protection of a given indicator by the addition of agents that serve as free radical acceptors (FRA), which compete with the indicator for the free radicals produced by the action of ionizing radiations. This phenomenon is illustrated in Equations 10 and 11:

$$\underset{\text{(active)}}{\text{Enzyme}} + OH^{\cdot} \longrightarrow \underset{\text{(inactive)}}{\text{Enzyme-oxidized}} + OH^{-} \quad (10)$$

$$\underset{\text{(active)}}{\text{Enzyme}} + OH^{\cdot} + \text{FRA} \longrightarrow \underset{\text{(active)}}{\text{Enzyme}} + OH^{-} + \underset{}{\text{FRA-oxidized}} \quad (11)$$

Another example of the protection of materials from the effects of ionizing radiations was obtained in studies with histidine.HCl, one of the essential amino acids. Figure 5 illustrates the dilution effect noted when this

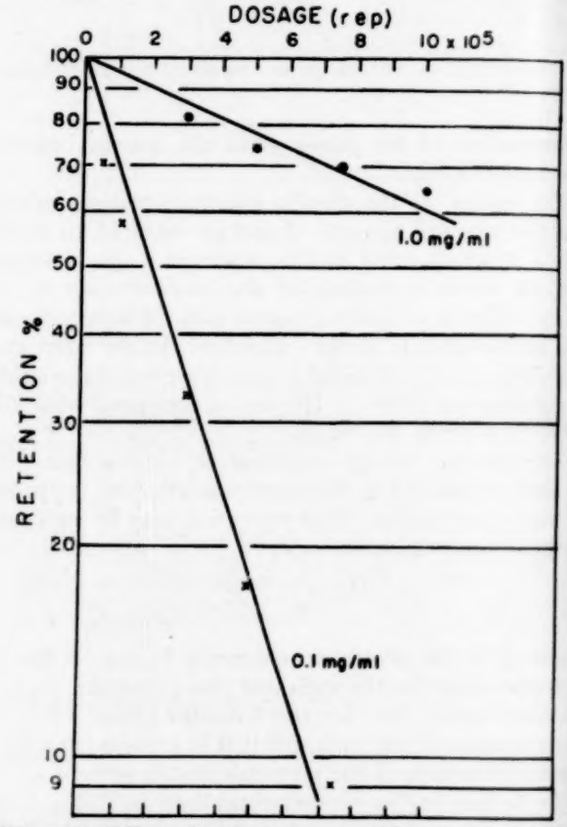

Figure 5. Effect of cathode rays on different concentrations of histidine.HCl in aqueous solution.

amino acid was irradiated in aqueous solutions of different concentrations, an effect similar to that noted with pepsin. Figure 6 illustrates the protective effect of D-isoascorbic acid on histidine. The protective quotients in this instance (Table 2) indicate that D-isoascorbic acid was a good protective agent for histidine, although not so good as sodium D-isoascorbate was for pepsin.

A further illustration of the protective action of free radical acceptors was afforded by the application of this technique to the prevention of hemolysis of red blood cells. In observations made in 1948 *(13)*, it was found that 5% suspensions of washed red blood cells in physiological saline were hemolyzed by supervoltage cathode rays. In the study reported here, this work was repeated with a control suspension of red blood cells and with suspensions to which sodium sulfite and sodium D-isoascorbate had been added (Figure 7). In all instances the concentration of red blood cells in the suspension was 5% (vol./vol.). Hemolysis was measured by spinning down a 15-ml. suspension of cells in a centrifuge tube and measuring the optical density of the supernatant in a colorimeter at 530 mμ.

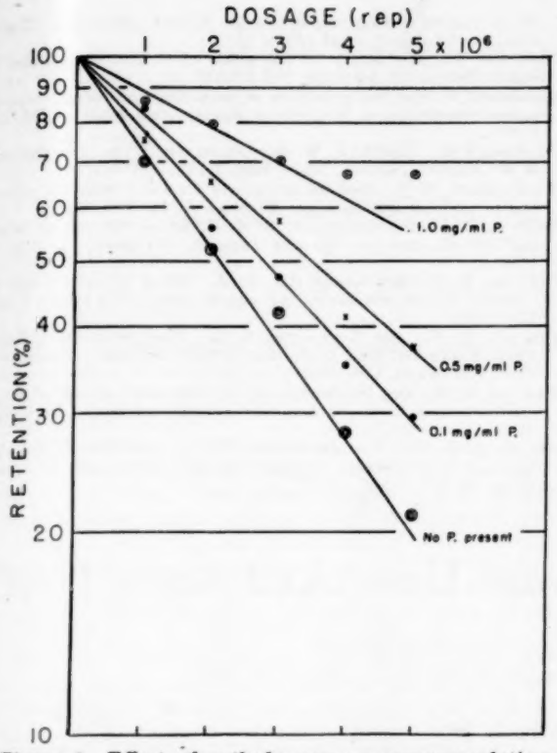

Figure 6. Effect of cathode rays on aqueous solutions of histidine.HCl (1.0 mg. per ml.) in the presence of varying concentrations of D-isoascorbic acid.

With the control suspension, hemolysis began at a dose level of approximately 250,000 rep. Addition of 1% (wt./vol.) of sodium sulfite delayed hemolysis until a dose level of 1,500,000 rep was reached, and addition of a like percentage of sodium D-isoascorbate delayed hemolysis until a dose level of 2,000,000 rep.

A more valid comparison might be the dose levels at which 50% hemolysis occurred in these instances, which were:

Control	1,000,000 rep
Sulfite	2,250,000 rep
Sodium D-isoascorbate	3,500,000 rep

With the addition of these protectors it is possible to prevent hemolysis of suspensions of red blood cells in vitro when exposed to cathode rays at doses below about 2.25 to 3.5 million rep. The potentialities and implications of this finding for in vivo studies are obvious. This technique makes the possibilities of sterilizing bovine blood for food specialty items and for animal consumption become more realistic, because pathogenic non-sporulating organisms are destroyed by cathode ray doses of 1,000,000 rep or less and the hemolytic effect of such doses can be avoided by means of this biochemical technique.

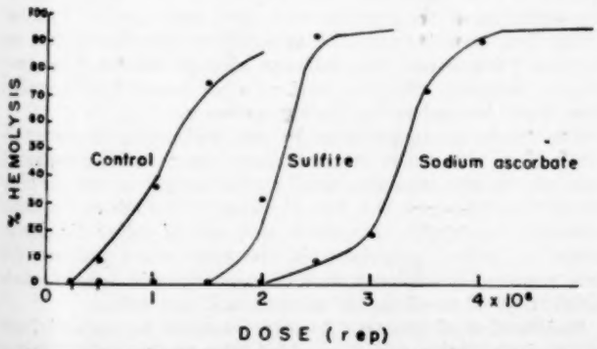

Figure 7. Effect of cathode rays on the hemolysis of red blood cells (bovine) in physiological saline solution (control) and in solutions to which sodium sulfite and sodium D-isoascorbate had been added.

The authors have evaluated the possible protective action against the side effects of cathode rays afforded by addition of a number of free-radical acceptors to several foods. Chopped meat products (cured) with which 0.5% by weight of ascorbic acid or one of its derivatives was intimately mixed prior to irradiation were indistinguishable from control samples in color, flavor, texture, and odor even after exposure to cathode ray doses of 2×10^6 rep (irradiation at room temperature). The control samples (no FRA added) showed a change in color after a dose of only 100,000 rep, which is far below the sterilizing dose.

The protection of a flavor component by the addition of a free radical acceptor (FRA) may be illustrated as follows:

$$\text{Flavor molecule} + OH^{\cdot} \longrightarrow \text{Oxidized off-flavor} + OH^{-} \qquad (12)$$

$$\text{Flavor molecule} + OH^{\cdot} + FRA \longrightarrow$$

$$\text{Flavor molecule} + OH^{-} + \text{FRA-oxidized} \qquad (13)$$

According to present regulations of the Federal Food and Drug Administration, it is permissible to add only a few compounds to foods. Among these compounds, which are harmless, are ascorbic acid and its derivatives mentioned in this report.

It is believed that this biochemical approach is a most logical one to prevent the undesirable side reactions produced by ionizing radiations without increasing the sterilization dose, as is necessary when inert atmospheres (such as nitrogen) are used. Further research on the application of this technique to other foods and to drugs is in progress.

SUMMARY

The reactions of solutes with free radicals produced by ionizing radiations (3 m.e.v. cathode rays) can be prevented by mixing with the solutes certain compounds that will compete for the free-radicals and thus protect the solutes against undesirable side effects.

That the mechanism of protection is not simply an oxidation-reduction effect is amply illustrated by the results obtained when niacin was used as the free-radical acceptor. Niacin is resistant to oxidation.

Observations were made with the enzyme pepsin, the amino acid histidine.HCl, suspensions of red blood cells, and samples of chopped (cured) meat products. It was demonstrated that ascorbic acid and its derivatives (D-isoascorbic acid and sodium D-isoascorbate), niacin, and sodium sulfite, designated as free radical acceptors, all had a protective action when combined with one or the other of the solutes.

Hemolysis of red blood cells in vitro at sterilizing doses of radiation was prevented, and the color, flavor, texture, and odor of chopped meat products remained unchanged when they were irradiated in the presence of a free radical acceptor.

Acknowledgments

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We wish to thank Professor J. G. Trump and Mr. K. A. Wright of the Department of Electrical Engineering, Massachusetts Institute of Technology, for their cooperation in placing the electrostatic generator at our disposal for this study.

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Natural Flavor Retained in New Frozen Uncooked Apple Pulp*

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A new process for the preparation of uncooked frozen apple pulp makes it possible to retain the fresh apple flavor. The essential steps in the process consist of 1, treating apple slices with a combination of antioxidants (sodium chloride, ascorbic acid, and sodium bisulfite) which behave synergistically; 2, freezing; and 3, pulping treated apple slices in the frozen state.

Minute concentrations of SO_2 inactivate polyphenolase enzyme more efficiently in presence of ascorbic acid and sodium chloride.

The new frozen apple pulp was found to be an excellent base for fresh apple-flavored sauce, sherbet, and ice.

A new type of uncooked frozen apple pulp which retains the natural fresh apple flavor has been developed and its home preparation briefly described by Johnson *(1)*. Essentially, the new process consists of treating apple slices with a combination of antioxidants, freezing, and then pulping the slices in the frozen state. Upon the addition of about 10% sugar, this pulp can be used in the same manner as the conventional frozen apple sauce. It is very smooth in texture and possesses the characteristic fresh apple flavor. After the addition of sugar, the pulp is completely thawed, preferably deaerated, and then packaged and refrozen. The conventional method of preparing frozen apple sauce involves a heat treatment, usually steam scalding of slices prior to pulping and addition of sugar. This mild heat treatment alters the delicate fresh flavor of the apple.

In the new process the heat treatment of apple slices is omitted. Instead, the slices are dipped in a bath containing a combination of antioxidants consisting of sodium chloride, ascorbic acid, and sodium bisulfite. Johnson and Guadnagni *(2)* described the synergistic effects obtained by the use of the above antioxidants in a patent on prefreezing treatment for fruits.

An important fact about this new apple pulp or sauce is the quality of apple sherbet and ices which can be made using it as a base. The present paper deals with the procedure with a view towards its commercial application.

EXPERIMENTAL METHODS

Details of process. The apples are peeled, cored, and sliced in the usual manner. It is best to slice the apples into 12 slices. Smaller apples may be sliced into 8 pieces. The slices are then dipped 1½-2 minutes in a bath consisting of 1% sodium chloride (salt), 0.3 to 0.4% ascorbic acid, and 300 p.p.m. of sulfur dioxide (SO_2) as sodium bisulfite. After dipping, the slices are drained and then frozen. Because the slices are to be subsequently pulped in the frozen state, it is convenient to freeze the slices on trays covered with waxed paper and then pack the frozen slices in a loose pack which will be more convenient for future pulping. The regular 30 pound tin or even a larger container may be used. If desirable, the slices may be packed after dipping and then frozen. This procedure makes it more difficult to break the frozen slices apart before pulping.

The ascorbic acid is stable in the above dipping bath. The chloride and sulfite ions prevent enzymatic oxidation and retard auto-oxidation of the ascorbic acid. The bath can be used for a long time provided that the antioxidants are replenished as they are being used. The bath can also be filtered when the occasion demands. Stainless steel or other non-corrosive equipment should be used in the dipping operation.

The uptake of antioxidants by the apple slices is approximately 10 to 12% of the concentration of the bath. For example, when the weight of apples equal to the weight of the dipping solution is used, there is a loss of about 10 to 12% of the antioxidants. The uptake of ascorbic acid and of sulfur dioxide is shown in Table 1. Analyses of the apple slices indicate the same result. Slices dipped in 0.4% ascorbic acid solution contained about 40 to 45 mg. of ascorbic acid per 100 g.

Modification of process. Ascorbic acid can be omitted from the dipping solution and then added later to the pulp with the sugar. The sugar containing the ascorbic acid should be added immediately after pulping, preferably before the pulp completely thaws. About 140 to 170 mg. of ascorbic acid per pound

* Scientific Journal Series No. 382, Colorado Agricultural Experiment Station, Colorado A and M College, Fort Collins, Colorado.

TABLE 1

Uptake of ascorbic acid and sulfur dioxide by Jonathan apple slices dipped in a 12 liter (3.17 gallon) bath containing 1% salt, 0.5% ascorbic acid, and 300 p.p.m. SO_2 ($NaHSO_3$)

Apple slices	Ascorbic acid removed from bath	Uptake of ascorbic acid		Uptake of sodium bisulfite	
lb.	*g.*	*mg. per lb.*	*mg. per 100 g.*	*mg. per lb.*	*p.p.m. SO_2*
24	5.9	246	54	18	24
72	15.3	212	46	....	....
96	21.2	221	48.5	17.2	23

of apple pulp should be added. This procedure is somewhat more economical than when ascorbic acid is used in the dipping solution, and is just as effective for treating the apples to prevent enzymatic browning.

After the addition of the sugar-ascorbic acid mixture to the pulp it is completely thawed. The sugared plp is then deaerated, packaged, and frozen. The sugared pulp can be used directly in such products as sherbets and ices. Then deaeration of pulp is not necessary. The pulp can even be stored for a few days at ordinary refrigerator temperatures. If, however, the sugared pulp is to be stored in the frozen state for several months, it is advisable to deaerate the product. When apple slices are pulped in the frozen state and thawed, a considerable amount of air is occluded in the product. This air is detrimental to the delicate fresh apple flavor even when stored at —10° F. (—23.5° C.) for several months. This will be discussed more in detail under the section on effectiveness of antioxidants and stability of flavor.

Methods of pulping. In all our experimental work, an ordinary type of meat grinder was used for pulping. The grinder attachments were precooled in freezer room before pulping. This type of grinder has a somewhat limited capacity but there are other comminuting machines of similar types and with much greater capacity that can be used.

Methods of analysis. Ascorbic acid was determined essentially by the method of Loeffler and Ponting *(3)*. Oxalic acid (0.25%) was used as the extractant.

Sulfur dioxide content of the apples was estimated by the method of Ponting and Johnson *(4)*. A 0.01*N*. iodine solution was used for titration.

Sulfur dioxide content of the dipping solution was determined by titrating two 10 ml. aliquots of solution, acidified with 1 ml. of 6*N*. HCl, with 0.02*N*. iodine solution. Then 1 ml. of 40% formaldehyde was added to one of the acidified aliquots and allowed to stand for 10 minutes before titrating. Sulfur dioxide content was calculated from the difference between the two titrations. Sulfur dioxide is bound by the formaldehyde. Ascorbic acid can be calculated from the titration of the aliquot containing formaldehyde. It is rather difficult to determine accurately the small amount of sulfur dioxide in presence of the relatively high concentration of ascorbic acid.

Since the total titration for the combination of ascorbic acid and sulfur dioxide can easily be determined, it can be used for following the loss of the antioxidants. When a 10% drop in titration has occurred, the antioxidants can be replenished by the addition of 10% of the original amount of each of the antioxidants (sodium chloride, ascorbic acid, and sodium bisulfite) added.

When ascorbic acid is omitted from the dipping solution the sulfur dioxide content can be estimated by titrating an acidified aliquot of the solution.

The method of Ponting and Joslyn *(5)* was used for the estimation of polyphenolase activity. The method used employs the rate of oxidation (browning) of catechol. A 3-minute reaction time was used. Enzyme activity is expressed in log T units or optical density produced per minute per g. of sample at 25° C. An Evelyn colorimeter with a 420 mμ filter was used to follow rate of color formation.

The following procedure was used to obtain the rate of browning apples: A 50 g. sample of frozen slices was weighed in a Waring blendor cup. Then 500 ml. of distilled water was added and the mixture was blended for 1 minute. It was then filtered through a milk cotton filter on a Buchner funnel with the aid of suction. An approximate 20-ml. sample was poured into an Evelyn colorimeter tube. This tube was placed in a water bath at 77° F. (25° C.) for about 15 seconds. The tube was then placed in the colorimeter which was adjusted to read slightly over 100% transmission. As soon as the galvanometer reading reached 100, the stop watch was started and the rate of color formation was followed for 10 minutes. The operation was carried out in such a manner that the galvanometer readings were started at the beginning of the fourth minute. A 420 mμ filter was used in this determination.

Jonathan apples were used in the experimental work except when otherwise indicated.

EXPERIMENTAL RESULTS

Effectiveness of antioxidants. Synergistic effects are obtained by using a combination of sodium chloride, ascorbic acid, and sodium bisulfite for controlling enzymatic browning. It is possible to use such small quantities of sulfur dioxide that no taste from that substance is detectable and yet in case of Jonathan apples it is possible to obtain complete inactivation of the polyphenolase enzyme. We found the reason for this to be that more polyphenolase enzyme can be inactivated by a very small amount of sulfite in the presence of relatively high concentration of ascorbic acid than by the same quantity of sulfite alone. Ponting and Johnson *(2)* showed that the polyphenolase system can oxidize sulfur dioxide. This oxidation of sulfite takes place during inactivation of the enzyme by the sulfite. If the SO_2 concentration is very low, little inactivation of the enzyme takes place because all the sulfite will be oxidized before it can inactivate the enzyme. With increased amount of sulfite the inactivation rate is increased and the enzyme will be inactivated before much of the sulfite is oxidized. This will leave a relatively high residual amount of SO_2. It appears that ascorbic acid is preferentially oxidized by the polyphenolase system and thus protects sulfite from enzymatic oxidation. The sulfite is then free to do more inactivation of the enzyme. As the enzyme is inactivated, the ascorbic acid oxidation is also retarded. Experimentally, this was actually found to be the case. The results are illustrated in Table 2. The analyses of the frozen apple slices were carried out after 3½ months of freezing storage at —5° F. (—20.6° C.). The original polyphenolase activity of the apple was 0.75 log T units per minute per g. The residual enzyme activity of the frozen slices was 0.22 log T units. Immediately after defrosting the pulp (8% sugar was added) the enzyme activity had dropped to 0.146 log T units. It continued to drop and decreased to 0.109 after 3 hours, 0.055 after 24 hours at 50° F. (10° C.) and finally to 0.032 log T units after 2 days at 50° F. In the same period of time ascorbic acid dropped from 47.8 mg. per 100 g. to 24.9 mg. per 100 g.

Table 3 shows what happens to the enzyme activity when ascorbic acid was omitted from the dipping solution. Jonathan apple slices were dipped in a solution of 1% salt and 300 p.p.m. of SO_2. The original enzyme activity was the same as above. Immediately after pulping and still in the frozen state the pulp had an enzyme activity of 0.24 log T units per minute per g. Merely by dipping the apples in the sulfite solution, there was a loss of about 60 to 70% of the enzyme activity. The

TABLE 2

Polyphenolase activity and ascorbic acid content of Jonathan apple sauce containing 8% sugar. The slices were dipped in a solution containing 1% salt, 0.5% ascorbic acid, and 300 p.p.m. of SO_2 (as $NaHSO_3$).

Conditions	Ascorbic acid mg. per 100 g.	Polyphenolase activity log T units per minute per g.
Untreated frozen slices	.....	0.74
Treated frozen slices (before pulping)	47.8	0.22
Immediately after defrosting pulp and sugar (about one hour)	44.8	0.146
3 hours after defrosting	.....	0.109
Stored at 50° F. for 24 hours	30.1	0.055
Stored at 50° F. for 48 hours	24.9	0.032

apple slices had a residual SO_2 content of approximately 15 to 20 p.p.m. of SO_2. It will be noticed that even after storage of the pulp at 50° F. (10° C.) for 1 day, no further inactivation of the enzyme took place.

To a new sample of freshly pulped apples approximately 50 mg. per 100 g. of ascorbic acid was added while still frozen. Immediately after defrosting the enzyme activity was 0.24 log T units per minute per g.

TABLE 3

Effect of adding ascorbic acid to apple pulp made from frozen Jonathan apple slices dipped in a solution containing 1% salt and 300 p.p.m. of SO_2 as ($NaHSO_3$).

Conditions	Ascorbic acid mg. per 100 g.	Polyphenolase activity log T units per minute per g.
Frozen pulp	---	0.24
Immediately after defrosting	---	0.23
Stored for 24 hours at 50° F.	---	0.24
8% sugar plus 50 mg. ascorbic acid added to frozen pulp immediately after defrosting	43	0.24
Same, stored for 24 hours at 50° F.	35.4	0.025

After 24 hours at 50° F. (10° C.), the enzyme activity dropped to 0.025, a very low value.

Table 4 shows results when ascorbic acid is added to apples which were untreated except for an immersion of the whole peeled and cored apples in 1% salt solution. Then 50 mg. of ascorbic acid per 100 g. of pulp was added with the 8% sugar immediately after pulping. The enzyme activity was 0.74 log T units per minute per g. After 22 hours at 50° F., there was no change in enzyme activity. A large loss in ascorbic acid took place. It has since been found that even the small amount of uptake of salt by the whole peeled apples reduces rate of ascorbic acid oxidation. With no salt, the oxidation of ascorbic acid would have been even greater than indicated in Table 4. (See section on the effect of sodium chloride.)

TABLE 4

Effect of adding ascorbic acid to apple pulp made from untreated slices. Eight % sugar plus ascorbic acid (50 mg./100 g.) were added to the apple pulp immediately after pulping in the frozen state.

Conditions	Ascorbic acid mg. per 100 g.	Polyphenolase activity log T units per minute per g.
Defrosted and stored at 50° F. for 3 hours	24.7	0.74
Stored at 50° F. for 22 hours	13.1	0.72

The above experiments show that it is possible to inactivate the polyphenolase enzyme with extremely small quantities of SO_2 in presence of sufficient ascorbic acid to protect SO_2 from enzymatic oxidation. Further experiments have been carried out to substantiate this finding. The uptake of SO_2 by apple slices dipped in 300 p.p.m. of SO_2 is very small. It is slightly over 20 p.p.m. After the slices dipped in a solution of 1% salt, 0.4% ascorbic acid, and 300 p.p.m. of SO_2 (as $NaHSO_3$) have been stored for a year or longer at —10° F. (—23.5° C.), the SO_2 content may drop to such a low value that no further inactivation of the residual enzyme activity takes place after pulping and thawing. For example, Jonathan apple slices treated as above and after 13 months of freezing storage had residual enzyme activity of 0.38 log T units when sampled in the frozen state. After pulping and two and one-half hours after thawing, the enzyme activity had not changed. Then 15 p.p.m. of SO_2 (as $NaHSO_3$) was added to the thawed pulp. After standing for 2 hours at 35° F. (1.7° C.) the enzyme activity had dropped to 0.12 log T units. A similar situation was found with Delicious apples. The enzyme activity of the frozen treated slices was 0.546 log T units. After pulping and defrosting and standing at 35° F. (1.7° C.) for 17 hours the enzyme activity dropped to 0.318 log T units. However, when 15 p.p.m. SO_2 (as $NaHSO_3$) was added at the time of defrosting, the enzyme activity was reduced to 0.022 log T units per minute per g. in the same length of time.

It was found that the inactivation reaction of the enzyme by the sulfite was more rapid at room temperature than at refrigerator temperature of 35° F. Frozen Jonathan apple slices treated by dipping in a solution of 1% salt and 300 p.p.m. SO_2 had polyphenolase activity of 0.241 log T units per minute per g. and contained 17 p.p.m. SO_2.

Immediately after pulping 10% sugar containing ascorbic acid (40 mg. per 100 g. pulp) was added in one case to the pulp. In a sample thawed in a water bath at 77° F. (25° C.), the enzyme activity was 0.087 log T units immediately after thawing and reaching a temperature of 77° F. After 4½ hours at 77° F., the enzyme was completely inactivated.

In another sample allowed to thaw to 35° F., the enzyme activity was still 0.24 log T units. After 4½ hours at 35° F. the enzyme activity was reduced to 0.153 log T units and after 24 hours the activity had dropped to 0.043 log T units per minute per g.

The Effect of Sodium Chloride

Besides stabilizing the ascorbic acid in the dipping bath, the sodium chloride adsorbed by the apple slices is sufficient to reduce enzyme action. This reduction of enzyme action reduces the rate of enzymatic oxidation of ascorbic acid during the time it takes the sulfite to inactivate the enzyme. This is important especially in cases where all the residual enzyme activity of the treated slices cannot be inactivated by the small amount of residual sulfite. Figure 1 shows how dipping the Jonathan apple slices in 1% salt solution reduces rate of enzymatic browning measured by the method described in the section on methods of analysis. Since the rate

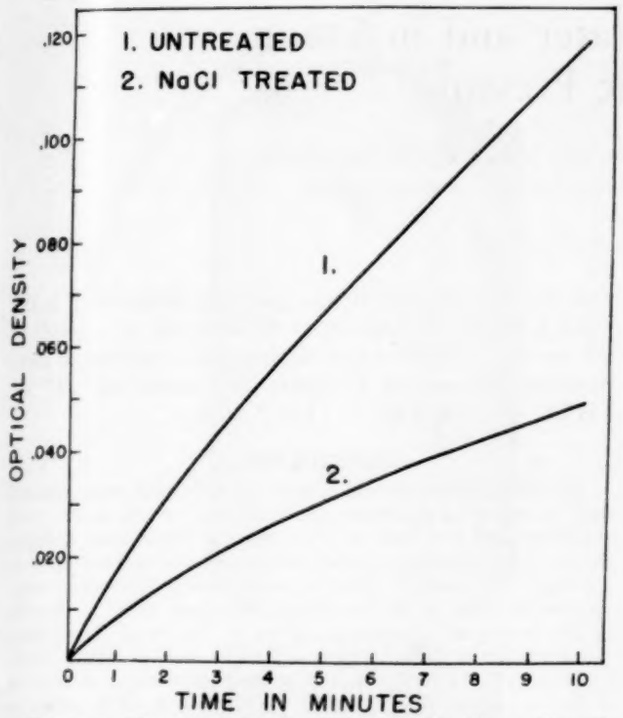

Figure 1. The effect of 1% sodium chloride dip on the rate of enzymatic browning of Jonathan apples.

of enzymatic browning is reduced, the rate of indirect enzymatic oxidation of ascorbic acid is also reduced. For example, when 40 mg. of ascorbic acid per 100 g. was added to freshly pulped untreated frozen Jonathan apples, only 20.7 mg. remained immediately after defrosting the pulp while 38.7 mg. of ascorbic acid remained in the sodium chloride-treated apples. After 18 hours at 35° F. (1.7° C.), the untreated sample had 1.9 mg. and the treated 18.1 mg. per 100 grams.

The effect of sodium chloride on the polyphenolase activity is reversible so when the enzyme activity is determined by the rate of catechol oxidation, it has no effect. The extract is greatly diluted for this test.

Stability of Fresh Flavor

It was found that the treated apple slices stored at freezing temperatures retained their fresh apple flavor better than the apple sauce (pulp plus sugar). This is apparently due to the deleterious action of air (oxygen) occluded in the pulp after thawing the frozen ground pulp. When the thawed pulp containing the added sugar was deaerated, the pulp retained the fresh apple flavor about equally as well as the treated slices. After 6 months of storage —10° F. (—23.5° C.) tasters were unable to tell the difference between apple sauce freshly prepared from treated frozen slices and apple sauce prepared from the same lot of treated slices, deaerated and stored for the same length of time. In the same period of time apple sauce not deaerated had noticeably lost fresh apple flavor. Deaeration also retards oxidation of ascorbic acid. If the apple sauce is to be stored for any length of time, deaeration of the sauce before packaging is recommended.

Only fully ripe fruit with a pronounced apple flavor should be used to make the best flavored products. Jonathan apples were found to give excellent products. For maximum flavor, it is essential that the apples be properly mellowed before processing. Excellent products were also obtained using mixture of Jonathan and some other varieties such as McIntosh, Wealthy, and Red, and Golden Delicious. It has been found desirable that Jonathan apples make up ½ to ¾ of the mixture.

Delicious apples when used alone gave a product which was too bland in flavor because of the low acidity.

Apple Sherbet and Ice

This apple pulp product was found to be an excellent base for a new type of apple sherbet and ice. These products possess the natural fresh apple flavor and also have a velvety smooth texture. Formulas to be used in a 6-quart ice cream freezer are given below for an apple sherbert containing 40% apple pulp and an ice containing about 60% apple pulp. The sherbet is whipped to about 50% over-run and the ice to about 100% over-run.

	Sherbet formula			Ice formula [b]		
	g.	*lbs.*	*oz.*	*g.*	*lbs.*	*oz.*
Apple pulp (with 10% added sugar)	2000	4	6.4	2000	4	6.4
Granulated sugar	820	1	8	495	1	1.5
High conversion corn sirup solids [c]	200	..	7	85	..	3
Milk	900	2	..	none	..	..
Water	450	1	..	285	..	10
Gelatin	22	..	0.8	14	..	0.5
Pure vanilla extract	5	1 teaspoon		5	1 teaspoon	

[b] Adapted from Velva Fruit formula developed by Western Regional Research Laboratory, U.S.D.A.

[c] Frodex (42 dextrose equivalent) was used but white corn sirup can be used. Allowance for water content of the sirup should be made.

All the ingredients except water and gelatin are first mixed. The gelatin is dissolved in the amount of cold water called for in the formula and heated to about 180° F. (82° C.). Then the gelatin solution is slowly poured into the above mixture and mixed well and poured into an ice cream freezer as in making ordinary ice cream or sherbet. The sherbet is whipped to about 50% over-run and the ice to about 100% over-run.

SUMMARY

The preparation of a new type of uncooked frozen apple pulp which retains the natural fresh apple flavor is described.

The prevention of enzymatic browning and the retention of fresh flavor are accomplished with the use of a combination of antioxidants (sodium chloride, ascorbic acid, and sodium bisulfite) which behave synergistically.

Apple pulp processed by this method serves as a suitable base for fresh apple-flavored sauce, sherbet, and ice.

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Comparative Heating Rates of Bentonite Suspensions in Jars Processed in Boiling Water and in Steam at Atmospheric Pressure[a]

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(Manuscript received March 5, 1952)

When 1 and 5% bentonite suspensions in pint and quart jars were processed in boiling water and in steam at atmospheric pressure, the heating medium did not produce significant differences in either the average or the maximal f_h values observed. The jars processed in steam heated at a significantly more uniform rate than those processed in boiling water. When processing times were calculated by the general method from the slowest-heating and fastest-cooling curves, differences between the processing times of steam and water runs were statistically non-significant. Steam and water consumption were lower when the jars were processed in steam and spray-cooled than when processed in boiling water and cooled with water.

Until approximately five years ago, acid foods packed in community canneries were usually processed in boiling water. Today, processing in steam at atmospheric pressure is becoming increasingly common. The availability of a new type processing vessel utilizing steam at atmospheric pressure as the heating medium is primarily responsible for this change. The purpose of the study reported here was to compare: a, temperature distribution in boiling water baths, as they are generally used in community canneries, and in this new type processing vessel, developed and designated by Ford *(7)* as an "atmospheric cooker"; and b, heat penetration into bentonite suspensions in jars processed in boiling water and steam. At least 3,000 Ford atmospheric cookers are in use at present. Further expansion of steam processing appears likely inasmuch as the atmospheric cooker and its operation are to be described in a forthcoming publication of the Production and Marketing Administration of the U. S. Dept. Agr.; accordingly, temperature distribution and heat penetration data may be helpful to others in establishing processing times or in supervising processing in community canneries.

The use of steam at atmospheric pressure has proved troublesome as a processing medium in the past because of the difficulty of thoroughly and consistently venting air from the processing vessel. The atmospheric cooker depends upon steam introduced at the top of the cooker to purge it of air. Various investigators *(3, 4, 6, 9, 10)*, have reported that retorts may be vented more readily when steam is introduced at the top rather than at the bottom of the retort.

McConnell *(8)* found that peach halves heated more rapidly in water than in steam at 212° F. (100° C.) whereas Roberts and Benjamin *(11)* observed the reverse in processing tomatoes. Townsend et al. *(12)* reported that there were no significant differences in the j and f_h values of suspensions of bentonite as a result of the heating medium when tin and glass containers were processed in retorts in steam and water at 240° F. (115.6° C.) and 250° F. (121.1° C.).

EXPERIMENTAL

The same equipment and system of replicating measurements used to measure temperature distribution in retorts at 10 p.s.i.g. steam pressure *(10)* were used in studying temperature distribution in the atmospheric cooker and in retorts operated at 0 to 5 p.s.i.g. steam pressure using top-inlet steam. The retort hookup was the same as that previously described *(10)*. A diagram of the atmospheric cooker is shown in Figure 1. The atmospheric cooker is made in two sizes. The smaller size is designated as a No. 2 cooker and is about equivalent in capacity to a No. 2 retort. The cover of the cooker is 18½ inches in diameter by 29 inches in height. The base pan is 20¼ inches × 5 inches; the basket is 16 inches × 21¼ inches. The basket is held above the bottom of the base pan by a rack which is 4 inches high. The legs of the rack project beyond the basket and thereby serve in addition as supports for the cover so that about ½ inch clearance exists between the bottom of the cover and the bottom of the base pan. The cover of the No. 3 cooker is 24 inches × 36 inches; the base pan 28 inches × 5 inches; and the basket 22½ inches × 25 inches. The basket rack is 4 inches high. The drainage outlet in the base pan of either size cooker allows about 1.5 inches of condensate to accumulate as a water seal. During the come-up period, the pressure of the incoming steam and the expanding air is sufficient to agitate the condensate violently with the result that the air escapes. By the time sufficient steam passes through the condensate to cause it to boil, the cooker is thoroughly vented. The steam may then be throttled so that only a small amount escapes through the water seal during the processing period.

Although the heating rates of some foods were actually determined, in most cases 1% and 5% bentonite suspensions were used for the comparative determinations between steam and water because less variation within treatments resulted than when foods were used. The bentonite was prepared according to Townsend et al. *(12)*. The cold points were determined experimentally for each size of jar and percentage bentonite. Each determination consisted of 5 jars containing 1% bentonite and 5 jars containing 5% bentonite, with one thermocouple located at the slowest heating spot in each jar. Two thermocouples were exposed directly to the steam or boiling water. One was placed in the center of the top layer of jars; the second was placed just below the jar basket upon its vertical axis. A minimum of 3 determinations each were made for the steam and water runs.

The thermocouples were of copper-constantan calibrated wire, B and S gauge No. 24, continuous from the hot junction to the potentiometer. Each thermocouple was introduced into the jar in a slight groove in a 3/16-inch wooden dowel which in turn was held in place by a compression fitting. A seal was effected by slipping a short piece of rubber tubing over the wire and dowel, doubling the tubing back on itself, and then compressiong the doubled portion in the socket part of the fitting with the compression nut. The other end of the rubber tubing was pinched tightly against the thermocouple wire with a screw-type pinch-clamp as additional protection against leakage. Leakage was rare. A Brown recording potentiometer was used to measure temperature.

[a] Journal Series Paper No. 3, Georgia (College) Agricultural Experiment Station, University of Georgia, Athens.

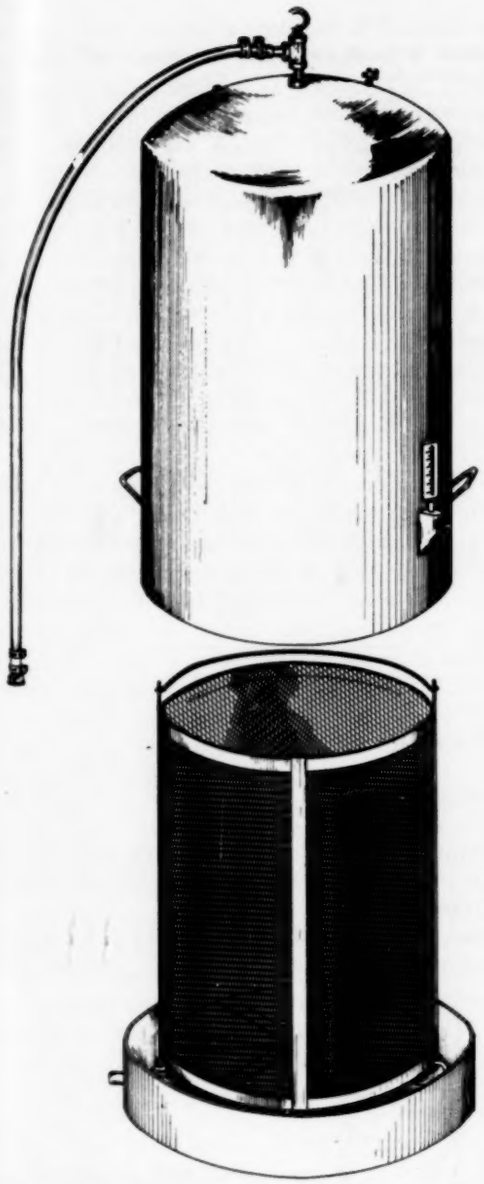

Figure 1. Pictorial diagram of atmospheric cooker showing base pan with basket supported in position on rack and cover which fits over basket.

When the heating rates of foods were determined, the cold point of each size jar and type of food was determined beforehand. Each determination consisted of 12 jars of the same size into which one thermocouple each was placed. The thermocouple assembly described above was generally used. However, in some instances thermocouples similar to those described by Townsend et al. *(12)* in which B and S No. 24 gauge copper-constantan calibrated wire is embedded in a molded bakelite rod were used. A small rubber gasket made the seal on the compression fitting. Temperature was generally measured with a Leeds and Northrup manually-operated potentiometer with an external reference junction, although the Brown recording potentiometer was used for some of the later determinations. When the manually-operated potentiometer was used, the 12 thermocouples were connected to a switch.

Whether the heating rates of bentonite or food were being determined, jars heated in the atmospheric cooker were transferred to a spraying chamber for cooling. A No. 1 solid cone spray,[b] delivering about 0.2 gallon of water per minute, was used during the first 0.5 to 1 minute of cooling; then both a No. 1 and a No. 5 spray,[c] the latter delivering about 1 gallon of water per minute, were used to complete cooling. When boiling water in an open retort was used as the heating medium, the jars were cooled with cold water admitted at the bottom of the retort, supplemented sometimes with a spray at the top. When shallow, rectangular tanks were used, the cooling water was introduced at the side of the tank.

[b] Catalogue No. G1, Spraying System Company, Chicago 24, Illinois.

RESULTS

Temperature uniformity in steam and water. The temperature distribution measurements showed that the atmospheric cooker was fully vented by the time the thermometer on the cooker registered 211° F. (99.4° C.), the temperature at which water generally boils in Athens, Georgia. The No. 2 cooker could be vented in 2, 6-10, and 15 minutes with a full load of jars at room temperature when steam at pressures of 65, 11-38, and 3 pounds was used. The come-up times of No. 3 cookers were generally only a few minutes longer. When No. 2 and 3 retorts heated with top-inlet steam were operated at 0 to 5 pounds pressure, the temperatures at all locations within the retorts were always within 0.5° F. (0.28° C.) of that of the retort thermometer at the start of the processing period.

Wider temperature differentials existed in the water than in the steam. When retorts were used for processing, a temperature differential of 4° F. (2.2° C.) often existed between the top and bottom of the retort although the water was apparently boiling throughout. This observation is in line with similar ones made with water-pressure processes at 10 pounds steam pressure in which temperature differentials of 9° F. (5° C.) were sometimes encountered with bottom-inlet steam unless air was used to circulate the water. Differentials of about 2° F. (1.1° C.) were encountered in shallow tanks heated with steam coils.

Heat penetration during steam and water processing. Comparisons of the heating rates of bentonite in jars heated in steam and water are shown in Table 1. Study of the differences in f_h values between the steam and water treatments using chi-square tests indicated that the heating medium caused no significant difference in heating rate. Neither the average nor the maximal f_h values differed significantly. Statistical analysis did indicate however that the variances of the steam runs were significantly less than those of the water treatments. The standard errors of the steam and water treatments were, respectively, 0.375 and 0.596 minutes for the 1% bentonite suspension and 1.57 and 1.69 minutes for the 5% suspension. Part of the greater variance of the water treatments may have been a result of excessive ebullition of the water causing agitation of the jars. Although efforts were made to prevent agitation of the jars, reasonably vigorous boiling was maintained to prevent, on the other hand, localized subboiling temperatures. If agitation did affect the rate of heat penetration to any extent, the effect was simply to increase the spread of the faster heating jars; it did not, as may be seen in Table 1, reduce the maximal f_h values of the water treatments below those of the steam treatments.

[c] Catalogue No. G5, Spraying System Company, Chicago 24, Illinois.

TABLE 1

Heat penetration into 1 and 5% bentonite suspensions in jars processed in steam (atmospheric cooker) and water (boiling water bath) at 211° F. (99.4° C.)[4]

Heating medium	Bentonite %	Initial temp., °F.	Pint jars (304 × 503)			Process for $F_{212} = 8.0$ (General method)	Heating F_{212}	Cooling F_{212}
			Come up time, minutes	j	Max. f_h value, observed			
Steam	1	82.5	10.0	0.44	11.4	22.0	7.10	0.90
Water	1	80.0	2.5	0.70	11.8	24.8	7.14	0.86
Steam	1	75.0	15.0	0.52	11.3	15.0	7.38	0.62
Water	1	105.0	21.0	0.64	16.8	16.0	7.55	0.45
Steam	1	132.0	2.0	1.90	10.2	18.0	6.89	1.11
Water	1	136.0	0.0	1.23	12.0	21.8	7.63	0.37
Steam	5	75.0	3.0	1.72	50.0	78.0	6.80	1.20
Water	5	76.0	8.0	1.42	52.0	76.5	7.30	0.70
Steam	5	129.5	12.0	1.20	53.6	59.2	6.36	1.64
Water	5	141.5	21.0	0.66	55.5	38.5	6.57	1.43
Steam	5	140.0	4.0	1.58	52.2	64.0	7.21	0.79
Water	5	136.5	0.0	1.53	55.5	70.0	7.50	0.50
Quart jars (400 × 609)								
Steam	1	72.0	1.0	1.65	14.5	31.2	7.32	0.68
Water	1	67.5	0.0	1.34	14.0	30.5	7.23	0.77
Steam	1	136.0	1.0	1.34	13.0	24.5	6.83	1.17
Water	1	137.0	1.0	1.17	15.3	27.0	7.65	0.35
Steam	5	75.0	1.0	1.47	84.0	116.5	6.77	1.23
Water	5	78.0	0.0	1.54	83.0	116.8	7.70	0.30
Steam	5	133.0	1.0	1.60	86.0	99.0	7.42	0.58
Water	5	138.0	1.0	1.64	84.0	98.0	7.68	0.32
One-half gallon jars (411 × 900)								
Steam	1	85.0	1.0	1.46	18.0	32.5	6.74	1.26
Steam	1	134.5	1.0	1.31	22.5	36.3	7.10	0.90
Steam	5	76.0	3.0	1.66	123.0	165.3	7.79	0.21
Steam	5	137.0	1.0	1.58	126.5	148.0	7.63	0.37

[4] Values for each treatment based upon a minimum of 3 determinations using 5 jars per determination.

When statistical confidence limits were applied to the f_h values within each treatment, the maximum f_h value actually observed frequently exceeded the 0.1% confidence level (actually the 0.05% level since the use of the upper limit only constitutes a one-tailed test). This observation is contrary to the finding of Esselen, Anderson, Ruder, and Pflug *(5)* who reported that actual and calculated limits of the heating rates of pickles agreed reasonably well. As may be seen from the standard errors listed above, variations in the heating rates were generally moderate.

Calculated processing times. When processing times having a F_{212} value of 8.0 were calculated by the general method *(2)* from the slowest-heating and fastest-cooling curves, differences between the processing times of water and steam runs were statistically non-significant. A lethality of 8.0 was used to permit comparisons of data on the two-piece metal lid, home-type canning jar with data reported by Townsend et al. *(12)* for commercial containers of approximately the same size. Although the percentage lethality derived from the cooling curve was as high as 20.5% of the total in some cases, in others it was as low as 2.7%. One reason the cooling period contributed so little to the lethality is evident from Figure 2 in which it may be seen that the temperature of the bentonite sometimes fell precipitously out of the lethal range as soon as cooling was started. Because home-type canning jars can vent during processing, high vacuums may ensue which, in turn, may lead to vigorous boiling during cooling when the headspace steam condenses. Townsend et al. *(12)* found that the contents of jars occasionally cooled faster than those of cans although the latter heated faster. They attributed the rapid cooling to the contents being stirred by "boiling." Boiling during cooling was not confined to bentonite suspensions. Foods exhibited similar cooling curves.

When processing times for pints calculated by the general method *(2)* and by Ball's method *(1)* were

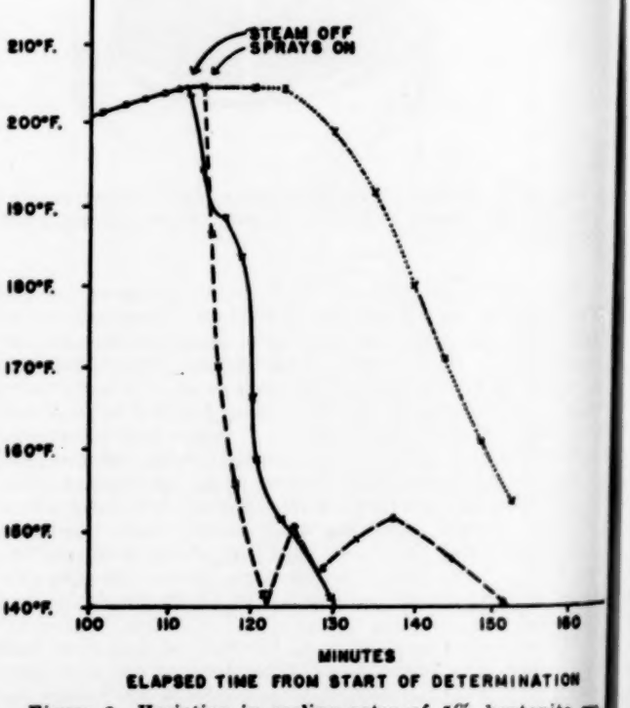

Figure 2. Variation in cooling rates of 5% bentonite suspension in quart jars, initial temperature 80° F. (26.67° C.). Solid and broken-line curves show typical cooling rates of bentonite boiling vigorously during cooling; dotted-line curve shows cooling rate when boiling did not occur.

compared, a very highly significant difference existed between the two methods irrespective of whether the cool correction suggested by Townsend et al. *(12)* for a No. 303 jar (a jar of about the same dimensions as a pint home-canning jar) was employed. An *e* value of 42% was used. The discrepancy noted may not be of practical importance because the differences were not numerically great and the particular lethality value used is probably excessive for most acid foods; nevertheless, application of the procedure outlined by Townsend et al. *(12)* for evaluating the cool correction needed and the portion of the come-up period which contributes to lethality to the limited data of this study indicated that the correction factors applied to No. 303 jars processed at 240° F. (115.6° C.) or 250° F. (121.1°C.) may not apply to pints processed at 211° F. (99.4° C.). Since there are no generally accepted levels of lethality necessary for various acid foods, the discrepancy was not studied further because at other lethality levels possible differences might be insignificant. For quarts the difference between the two methods of calculation was not statistically significant. An *e* value of 42% and the cool correction for a No. 2½ jar derived by Townsend et al. *(12)* were used in the formula method *(1)*. For both pints and quarts and the two methods of calculation, the variances of the processing times within each treatment were homogeneous.

Confidence limits were also established for all the processing times within each treatment using the heating and cooling curve of each individual jar to see whether the upper levels would have any predictive value as far as possible maxima were concerned and to see whether such limits would include the process based upon the composite slowest-heating fastest-cooling curve. In many cases the processing times based upon the composite curve exceeded the upper 0.1% confidence level. The statistical evaluation thus merely indicated that the probability of any individual jar requiring a process in excess of that derived from the composite curve generally would be less than one chance in two thousand. The coefficients of variation for the steam and water processing times were respectively 5.12 and 7.06% for the individual determinations within each treatment.

Heat penetration rates for foods. Heating rate data are shown in Table 2 for the foods studied. The heating rates listed are the slowest rates observed. During the 1947 season tomatoes always yielded straightline curves on semi-logarithmic coordinates. During 1948 broken heating curves always resulted. The heating rates of acid foods were very erratic which agrees with the findings of other investigators *(8)*.

Steam and water consumption. Steam and water consumption were also measured when some of the foods were processed in the atmospheric cooker and in boiling water. About 37% less steam was generally consumed when the atmospheric cooker was used than when boiling water processing was employed. Water consumption was usually lower when the atmospheric cooker and spray cooling were used than when water processing and cooling were used although the exact relationship varied depending upon the rate of spraying or water cooling and the number of jars being cooled. Unless the volume of food being processed is sufficient to require continuous use of the boiling water bath, the atmospheric cooker is generally more economical of both water and steam.

SUMMARY

1. Temperature distribution in the Ford atmospheric cooker was excellent. By the time the thermometer on the cooker registered 211° F. (99.4° C.) (the temperature at which water usually boils in Athens, Georgia), the cooker was at 211° F. (99.4° C.) throughout.

2. Good venting characteristics were observed when retorts heated with top-inlet steam were operated at atmospheric pressure or at steam pressure up to 5 pounds.

3. Temperature differentials of 4.0° F. (2.2° C.) and 2.0° F. (1.1° C.) were frequently observed when retorts and shallow tanks were used for boiling water processing of acid foods.

4. No significant differences in f_h values or calculated processing times for 1 and 5% bentonite were discernible as a result of jars being processed in boiling water or steam at atmospheric pressure.

5. Bentonite suspensions heated at a significantly more uniform rate in jars processed in the atmospheric cooker than in boiling water baths.

6. Steam and water consumption were generally lower when the atmospheric cooker was used than when boiling water baths were used to process the jars.

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The technical assistance and suggestions of W. Carlos Mills, Lionel W. Mills, Charles M. Balkcom, and Roger W. Ford is

TABLE 2

Heat penetration into pint (304 x 503) and quart (400 x 609) jars of acid foods processed at 211° F. (99.4° C.) in steam (atmospheric cooker) or in boiling water bath

Product	Jar size	Number of jars	Heating medium	Initial temp., ° F.	Come up time, minutes	j	Max. f_h value, observed	f_2	x_{bh}
Applesauce	Pint	72	Steam	179	1	3.06	44.0	.....	.....
Peaches, quarters	Quart	59	Steam	95	6	1.16	73.0	.....	.....
Peaches, quarters	Quart	60	Water	95	19	0.99	66.0	.....	.....
Pears, halves	Pint	48	Steam	75	5	1.69	43.0	.....	.....
Pears, halves	Pint	48	Water	74	5	1.47	39.5	66.0	34.3
Pimientos, whole, brine pack	Pint	23	Water	93	4	1.46	33.3	.....	.....
Sauerkraut	Pint	24	Steam	147	2	1.77	40.3	.....	.....
Tomatoes	Pint	105	Steam	89	3	1.48	45.0	19.0	39.0
Tomatoes	Pint	101	Water	83	4	1.44	58.0	27.5	40.0
Tomatoes	Quart	97	Steam	88	7	1.49	83.3	.....	.....
Tomatoes	Quart	103	Water	80	9	0.99	81.0	42.3	49.8
Tomato Juice	Pint	24	Steam	140	3	1.89	48.5	.....	.....
Tomato Juice	Quart	24	Steam	142	3	1.49	89.0	.....	.....

gratefully acknowledged. The authors thank Charles E. Turner and Roy J. McCraney of the Agricultural Engineering Department, University of Georgia, for drawing Figure 1.

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New Designs and Techniques for Organoleptic Testing[a]

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An experimental technique and analysis is presented for paired comparisons which is simple and efficient in the sense that relatively small numbers of observations are required. The method of testing and analysis is illustrated and necessary tables are included.

The analysis of data from experiments involving organoleptic testing has recently come under intensive study. It is known that many of the techniques now in use are unsatisfactory and that improved procedures are required. This paper illustrates a new theory applicable to many current problems in organoleptic testing where qualitative measures alone are valid and reliable. The method will be particularly useful in those problems involving the detection of differences among several treatments by sensory methods by a small panel of judges.

Heretofore, the best experimental testing of food products, clothing, tobacco, and the like, which require subjective measures, has involved the comparison of two products. In many cases such tests consisted of comparing an experimental product with a standard or with a competitor's product. Statistical analyses of sensory difference tests have been reviewed by Bradley and Duncan *(1)* and Bradley and Terry *(3, 4)*.

Experiments involving more than two products or treatments have had varying success. Many artifices have been employed without consistently achieving satisfactory results, although scoring methods and transformations of ranked data have been widely used *(8, 9)*. Particular difficulties are introduced in those experiments where it is impossible to rate, score, measure, or rank all of the treatments at one sitting. Incomplete block designs *(6)* partially alleviate these difficulties, but the method of scoring or the analysis of rank data is often unsatisfactory.

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In this paper a method of analysis is proposed for paired comparisons which is exact for small samples and which involves only extremely simple computations. The experimental design is well-known; the statistical analysis is new. The procedure has the advantage that it is extensible to incomplete block designs with more than two treatments appearing in a block, and these extensions are under development.

Other recent papers on paired comparisons are of supplementary interest *(10, 12, 16)*.

THE ANALYSIS OF SENSORY DIFFERENCE TESTS

One of the difficulties in usual experimentation involving sensory differences is the choice of a suitable scoring scale and the use of standards to coordinate scoring. Most analyses, when more than two treatments are compared, depend on the analysis of variance. The analysis of variance depends on 4 basic assumptions: 1, observations are independently distributed; 2, observations come from a normal population; 3, error variances are homogeneous; and 4, treatment and environmental effects are additive.

In sensory difference tests using scoring methods all of these assumptions are to some degree suspect, and the effects of their violation are not well understood. It appears that taste fatigue may introduce departures from Assumptions 1 and 3. A discrete scoring method without the use of standards usually leads to departures from Assumptions 3 and 4. One would require 2 spaced standards as a minimum to provide a uniform scale for a number of judges, and they introduce so much extra testing that fatigue develops more rapidly. The discreteness of a scale always leads to violation of Assumption 2, although this may not be too serious *(2)*, and some of the psychological effects of subjective judging discussed by Thurstone *(15)* appear to cause concern over the validity of Assumption 4.

Triangle and duo-trio tests have been developed in sensory difference testing, and a good review of these

methods was given by Peryam and Swartz *(13)*. These tests are useful when only two treatments are compared, and they involve an attempt to avoid the restrictive assumptions of analysis of variance and to provide a simple test procedure. Tables for these tests are readily available in many forms, and Davis and Terry *(7)* provided a form easily used by personnel of the small processing plant. When several judges perform triangle or duo-trio tests, estimated probabilities of correct pairing by judges are reliable measures of individual sensory acuity, and these estimates may be used in comparing the abilities of judges. This information has not been widely used. However, it is this concept that will be generalized and expanded in the method that is presented here.

The triangle and duo-trio tests are similar to various types of rank-order tests and lead into more complex analyses using ranks. When two products are to be compared, there are a number of rank-order tests available, and these have been used to only a very limited extent in sensory difference detection. Some of the reports on such lines are those by Wilcoxon *(17)*, Mann and Whitney *(11)*, and Terry *(14)*.

A new test procedure is now presented using ranked data which considers comparisons of more than two products or treatments. The test is flexible. In subjective testing the experimenter may assume a priori that the standards of judging are uniform or that they vary by judges, by time, or by both. That is, true treatment ratings may be considered to be constant throughout an experiment or to be functions of judges and of time. In experiments concerned with the detection of treatment differences, the latter alternatives are important. Certain test characteristics may be rather intangible and difficult to describe with the added difficulty that personal preferences may influence judges' decisions. Even in simpler cases the research worker may desire to forego the tedious procedure of training and coordinating judges. In these situations tests of treatment differences may still be performed and a measure of the agreement among judges obtained, although estimates of over-all treatment ratings are not usually meaningful.

The Assumptions of the Experiment

Now consider the notation required to describe the experiment and the assumptions involved. The assumptions will be shown to be less restrictive than those of analysis of variance and it follows that the technique may be applied to a larger class of experimental data involving paired comparisons.

Consider t treatments in an experiment using paired comparisons. First postulate that these treatments have true ratings (or, in some cases, preferences) $\pi_1, \pi_2 \ldots, \pi_t$ throughout the experiment although we shall later relax this condition and add a second subscript indicative of some other sources of variation such as judges or time.

A repetition of the paired comparisons design is defined to be a complete set of all pairs of treatments. Reserve n to denote the number of repetitions of the design, and use r_{ijk} to denote the rank of the i^{th} treatment in the k^{th} repetition of the pair in which treatment i appears with treatment j. Estimates of $\pi_1, \ldots, \pi_t$ will be denoted by $p_1, \ldots, p_t$, respectively.

The basic assumptions of the analysis are: 1, observations on pairs of treatments are independent; and 2, true treatment ratings $\pi_1, \ldots, \pi_t$ exist such that the probability that treatment i obtains top rating when compared with treatment j is $\pi_i/(\pi_i + \pi_j)$. For definiteness, the sum of the treatment ratings is fixed at unity.

These assumptions are considerably less restrictive than those of the analysis of variance. Independence is still required but this is the only assumption common to both methods of analysis. In the new analysis Assumption 2, immediately above, replaces the 3 remaining assumptions in the former.

In certain cases, as previously noted, repetitions of the design may be performed by different judges or at different times. We can then think of true ratings $\pi_{1u}, \ldots, \pi_{tu}$ existing for the u^{th} judge of a group of judges or on the u^{th} day of an experiment or for some similar classification. Methods of amalgamating the results for several groups will be illustrated.

For applied work it is usually only necessary that the experimenter be familiar with the basic assumptions of a test procedure and the mechanics of applying the test. The mathematical considerations are omitted here but have been given in a separate paper *(5)*.

The Description of the Experiment

The paired comparison design is technically a completely balanced incomplete block design. We provide the analysis for the incomplete block design with 2 treatments in each block. That is, in the case of t treatments $(T_1, \ldots, T_t)$ each repetition involves the rating of pairs $T_1T_2, T_1T_3, \ldots, T_{t-1}T_t$. The method of rating is simple: A judge rates the 2 unidentified elements of the pair identifying the one that contains more or less of the attribute in question (such as saltiness, rancidity, irritativeness, sweetness, oiliness, intensity of color, clearness of liquid, aroma, etc.). If an element of the pair has more of the attribute under study, the judge gives it a rank or a score of 1, if it has less, he records the value 2. The interpretation of the results is facilitated by tables which are available. The computation is in general limited to simple addition except in those experiments wherein the number of treatments or of repetitions exceeds that for which tables have been completed.

When more than one judge is used, two possible situations arise. Either the experimenter knows that his judges will always rate the samples consistently as a group or he is only certain that the judges are consistent as individuals. It is possible for two judges to distinguish differences among the treatments equally well but to be completely opposite as to the order of treatment ratings. This problem frequently arises with a new panel or with an old panel testing products very different from those previously used. For some attributes such as flavor and aroma it is inherently difficult to be certain that all of the judges agree on what constitutes more of the attribute and what constitutes less. Heretofore this anomalous situation has been an obstacle to interpreting results. In this paper a simple calcula-

tion will evaluate the test data even if the judges have substituted for "more of the attribute" the less precise measure, "preference."

PROCEDURE FOR ANALYSIS

The following outline of the steps required for the conduct and analysis of experiments using ranking in paired comparisons will be useful. The outline for simplicity is written for 3 treatments but the extension to larger numbers will be obvious. The analysis is described for 2 cases, (A) where tables are available, and (B) where approximate solutions must be used.

Procedure

Step 1. (Preliminary.) Select a panel. Decide on the basis of past performance, the results of preliminary tests, or a knowledge of the experimental material whether this panel is to be considered as (*P*) consistent as a group or (*C*) consistent as individuals.

Step 2. For each judge and for each repetition take 6 small containers and number or code them. Place 2 samples of each of the 3 treatments in separate, but exactly similar containers and record the identity of the containers. Pair the containers as follows:

Pair	I	II	III
Treatment	(T_1) (T_2)	(T_1) (T_3)	(T_2) (T_3)

Record each pair by the code numbers on a suitable score card in a random order. Now place these pairs before the judge in a random order together with the score card.

Step 3. A judge will test each pair recording on the score card the value one (1) to that member of the pair containing more of the attribute and the value two (2) to that member containing less of the attribute.

Step 4. Repeat steps 2 and 3 for the desired number of repetitions.

Analysis

Step 5. The experimenter will collect and decode the score cards, recording the ranks in the following table:

		T_1	T_2	T_3
Score Card	Pair 1 (T_1) (T_2)	—	—	x
No._____	2 (T_1) (T_3)	—	x	—
Judge_____	3 (T_2) (T_3)	x	—	—
	Total	—	—	—

Step 6. Add the ranks on each score card for each treatment. Then for each treatment add the treatment totals on all the score cards for each judge. These 3 numbers (for 3 treatments) constitute the test data for this experiment for any one judge.

A. The Method Using Tables

Step 7(P). If one has assumed that judges are consistent as a group, the analysis will be as follows. For each treatment add the ranks sums or treatment totals for all members of the panel. These sums constitute the test data for the group.

Step 8(P). Rearrange the ranks sums in ascending order, smallest on the left. Enter Table 1 under the total number, n, of repetitions made and find the entry agreeing with the rearranged rank sums obtained. These rank sums appear in the left hand columns of the table. The extreme right hand column shows the significance level of the experimental data. If that entry is less than the predetermined rejection level of significance (usually 0.05 or 0.01), the experimenter may conclude that treatment differences exist.

Step 7(C). If one has assumed that each judge is consistent as an individual but that the panel may not be consistent, a slightly more involved analysis follows. From Step 6, for each judge, rearrange the treatment rank sums in ascending order from the left. Enter Table 1 for the number of repetitions made by each individual judge and record the B_1 value corresponding to his observed treatment rank sums. Total the values of B_1 to obtain B_1' for the panel. In passing one may note the significance levels of the rank sums for each judge by examining the probability values in the right hand column as in Step 8(P).

Step 8(C). Enter Table 2 under the number of "equal groups" (usually number of judges) appropriate for the experiment and under the proper total number of repetitions and read the level of significance corresponding to the test value B_1' of Step 7(C). Enough values of B_1' are tabled for easy interpolation.

Treatment ratings $p_1, \ldots, p_t$ are entered in Table 1. They afford a method of comparing treatment effects if interpreted according to the explanations of the next section. Treatment ratings may be obtained for the panel under analysis (*P*) or for individual judges under (*C*). No treatment ratings for the panel are obtained under (*C*) due to possible contrary opinions among judges.

B. The Approximate Method

In some cases the number of repetitions in an experiment may exceed those for which tables are available. It is then necessary to amend the steps in A by substituting a calculation where the tables were previously consulted. If the number of repetitions exceeds 10, the following approximate tests should be acceptable.

Steps 7(P) and 8(P). Estimates may be obtained for $p_1, \ldots, p_t$ by solving equations of the form

$$\frac{2n(t-1)-\Sigma r_i}{p_i}-\frac{n}{p_1+p_i}-\ldots-\frac{n}{p_t+p_i}=0,\ i=1,\ldots,t,$$

using the notation previously defined and where Σr_i is the rank sum for treatment i obtained in Step 6. The solution of these equations is difficult and when required one might consult a mathematician.

In some special cases one may avoid the necessity of solving the equations for $p_1, \ldots, p_t$. These occur firstly when n and the test data of Step 6 (for example, for 3 treatments, Σr_1, Σr_2, Σr_3) are all divisible by the same integer c and n/c repetitions are given in Table 1. Then, values of p_1, p_2, p_3, may be read directly from that table for the entry $\Sigma r_1/c$, $\Sigma r_2/c$, $\Sigma r_3/c$ under n/c repetitions. Secondly, when not all $\Sigma r_1/c$, $\Sigma r_2/c$, $\Sigma r_3/c$ are integers but n/c is an integer, this set of numbers will fall between two sets of rank sums in Table 1 under n/c repetitions. Then approximate values of p_1, p_2, p_3 may be obtained by interpolation. The best approximations will be obtained when the smallest possible integer c is used.

When satisfactory estimates p_1, p_2, p_3, are obtained, B_1 may be computed immediately by substitution in the formula,

$$B_1 = n\log(p_1+p_2) + n\log(p_1+p_3) + n\log(p_2+p_3) - [2n(t-1)-\Sigma r_1]\log p_1 - [2n(t-1)-\Sigma r_2]\log p_2 - [2n(t-1)-\Sigma r_3]\log p_3$$

where common logarithms are used. For 3 treatments t has the value 3 in the above formula. The formula for more than 3 treatments will be obvious.

The approximate test corresponding to Step 8(*P*) is obtained by computing $\chi^2_{t-1} = (0.6932)nt(t-1) - (4.6052)B_1$, where the subscript $(t-1)$ denotes the degrees of freedom used to select the appropriate row in the table of chi square. Large values of chi square are indicative of true treatment differences.

Steps 7(C) and 8(C). When the data are to be combined under the assumption of Step 7(*C*), it is necessary to compute B_1 for each judge or division of the experiment. In sensory difference work it is unlikely that a single judge performs more repetitions than the values tabled. However, if this does happen, one must approximate the values of $p_1, \ldots, p_t$ and B_1 as described above. Values of B_1 are necessary for each division of the experiment. These values are summed to obtain B_1' as described in Step 7(*C*).

Very often B_1' will be based on more repetitions than the tables permit. To make the combined test, we compute

$$\chi^2_{g(t-1)} = (0.6932)nt(t-1) - (4.6052)B_1'$$

where g is the number of judges or divisions. Again large values of chi square are indicative of true treatment differences.

A TEST OF AGREEMENT

Two test statistics are available for over-all panel tests of treatment differences and the choice of the proper one depends on the a priori assumptions on the behaviour of the panel members noted at the begnining of this section. These statistics B_1 and B_1^c may be used to test the agreement among panel members. An approximate test is given by

$$\chi^2_{(g-1)(t-1)} = (4.6052)(B_1 - B_1^c)$$

This chi square has $(g-1)(t-1)$ degrees of freedom and large values indicate disagreement among panel members. B_1 always exceeds B_1^c.

TABLES AND THEIR INTERPRETATION

The main body of tables for this experimental procedure and analysis will be included in the mathematical paper *(5)* on this method. Listed in the present article are sections from both Tables 1 and 2 below to illustrate their form and interpretation. These tables are so chosen to be sufficient for the illustrative example which follows.

Table 1 gives the values of the likelihood ratio statistic B_1 and the likelihood estimates of the true treatment ratings p_1, p_2, p_3 together with the probabilities that B_1 will not be exceeded. The sums of squares of deviations (S. S.) for treatment rank sums are included. The n is the number of repetitions of the design. Parentheses contain combinations with equal values of B_1. Σr_i is the sum of ranks for treatment i.

TABLE 1

The distribution of the likelihood ratio for general alternatives [b]

Σr_1	Σr_2	Σr_3	p_1	p_2	p_3	S. S.	B_1	Probability
				$n = 5$				
10	15	20	1.0	0.0	0.0	50	0	0.0002
10	16	19	1.0	0.0	0.0	42	1.0866	0.0020
11	14	20	0.8000	0.2000	0.0			
10	17	18	1.0	0.0	0.0	38	1.4614	0.0057
12	13	20	0.6000	0.4000	0.0			
11	15	19	0.8108	0.1583	0.0309	32	2.2744	0.0112
11	16	18	0.8165	0.1228	0.0607	26	2.7669	0.0386
12	14	19	0.6376	0.3150	0.0474			
11	17	17	0.8182	0.0909	0.0909	24	2.9170	0.0569
13	13	19	0.4737	0.4737	0.0526			
12	15	18	0.6570	0.2488	0.0942	18	3.3725	0.1000
12	16	17	0.6656	0.1925	0.1419	14	3.6454	0.2464
13	14	18	0.5129	0.3780	0.1091			
13	15	17	0.5324	0.2993	0.1683	8	4.0344	0.4039
13	16	16	0.5385	0.2308	0.2308	6	4.1581	0.6053
14	14	17	0.4118	0.4118	0.1765			
14	15	16	0.4268	0.3253	0.2479	2	4.3987	0.9313
15	15	15	0.3333	0.3333	0.3333	0	4.5154	1.0000

[b] Extracted from Appendix A, *(5)*. "S.S." denotes the sums of squares of deviations for treatment rank sums.

In Table 1 treatment rank sums are listed in ascending order from the left for convenience since any permutation of these sums leads to the same conclusion relative to the hypothesis of no true treatment differences. One must, of course, be consistent in the association of treatments with the subscripts on the rank sums and p's of Table 1. [See Step 8(P).]

The interpretation of the estimated treatment ratings, p_1, p_2, p_3, requires some care. In general, pairwise comparisons are valid in the sense that when treatment T_i is compared with T_j, the probability that T_i is rated above T_j is estimated to be $p_i/(p_i + p_j)$. When interest is centered on the relative positions of more than 2 treatments, differences between logarithms ($\log p_i$) of estimated treatment ratings lead to valid judgments.

The statistic B_1 and the "Probability" of Table 1 have been discussed in Step 8(P). The column headed S. S. (the sum of squares of rank sums about their mean) was only included due to interest in sums of squares through association with analysis of variance.

Table 2 gives selected values of the combined likelihood ratio statistic, B_1^c, together with cumulative probabilities (levels of significance) associated with each tabular value. Only groups of equal size are considered. Thus if n is the total number of repetitions of the design and 3 groups are considered, each group contains $n/3$ repetitions.

TABLE 2

The distribution of the combined likelihood ratio for general alternatives [c]

B_1^c	P	B_1^c	P	B_1^c	P	B_1^c	P
			3 equal groups				
n is 6		n is 9		n is 12		n is 15	
0	0.001	2.5112	0.001	5.3727	0.001	8.0688	0.001
1.2042	0.016	2.7093	0.002	6.2191	0.005	9.0642	0.005
1.4984	0.030	3.3405	0.005	6.6031	0.010	9.4794	0.010
1.8063	0.041	3.7357	0.011	7.0209	0.021	9.8945	0.020
2.1005	0.101	4.1698	0.022	7.1379	0.026	10.0456	0.025
		4.5097	0.032	7.3265	0.030	10.1738	0.031
		4.5883	0.040	7.5495	0.042	10.4352	0.039
		4.7466	0.052	7.6341	0.049	10.5704	0.052
		4.9835	0.063	7.8146	0.059	10.6882	0.060
		5.0224	0.071	7.9079	0.068	10.8049	0.066
		5.2205	0.101	8.0181	0.083	10.9031	0.080
				8.0781	0.088	10.9594	0.090
				8.1351	0.100	10.9858	0.097

[c] Extracted from Appendix B, *(5)*. Table 2 is used in Step 8(C).

B_1^c is computed by adding values of B_1 for each group as obtained from Table 1. Only values significant at approximately the 0.10 level of significance or higher are recorded and these are selected for easy interpolation.

AN ILLUSTRATIVE EXAMPLE

The following example is hypothetical and designed to illustrate the new method as simply as possible. Some indication of the actual applications of the method are given in the conclusion. Since those experimental results deal with basic research in various fields, the findings of those experiments and the applications of this technique are more properly deferred for publication by the research workers involved. Results and implications of industrial applications are not available for publication.

Assume that samples are taken from 3 varieties of sweet potatoes (perhaps Nancy Hall, Virginian, and Puerto Rico) processed as canned sweet potato and prepared for tasting by a standard home-cooking method. The attribute under test is sweetness. There are 3 judges, who have had considerable experience with organoleptic tests with sweet potatoes, available for this experiment. It is believed, but not known, that the 3 judges are consistent as a group in the evaluation of the apparently simple attribute, sweetness. Proceed with the experiment following Steps 1-6 and with the analysis following the steps of Subsection A with the

Tables. It is possible to verify the assumption of group consistency by the test of agreement.

The summary of the test data for sweet potatoes of varieties A, B, and C for judges 1, 2, and 3 of Step 6 is as follows:

TABLE 3

Summary of test data in example to illustrate new method

Judge	Variety			
	n	A	B	C
1	5	19	12	14
2	5	19	11	15
3	5	18	16	11
Rank sum	15	56	39	40

To test the hypothesis that there are no varietal differences in sweetness, we must, on the assumption that judges are consistent as a group, follow Steps $7(P)$ and $8\ (P)$. In addition, the total number of repetitions, $n = 15$, is outside tabled values and the analysis falls under "Subsection B. The Approximate Method."

Divide the total row of the summary table by 3 and arrange the rank sums so divided in ascending order. Table 1 is used for interpolation and the entry is made under $n = 5$. The process of interpolation is shown in Table 4.

TABLE 4

Interpolation for treatment ratings for data given in Table 3

	Σr_1	Σr_2	Σr_3	p_1	p_2	p_3
Table Entry 1	13	14	18	0.5129	0.3780	0.1091
(Observed) /3	13	13⅓	18⅔	0.4933[d]	0.4418[d]	0.0714[d]
Table Entry 2	12	13	19	0.4737	0.4737	0.0526

[d] Obtained by linear interpolation.

The estimated values of p_1, p_2, p_3 add to 1.0065 and may be adjusted to add to unity by division yielding values 0.4901, 0.4389, and 0.0710, respectively. B_1 and χ_2^2 are computed from the formulas given and we obtain *

$$B_1 = 9.6199 \text{ and } \chi_2^2 = 18.09$$

This value of chi square is significant at the 0.001 significance level. It is concluded therefore that the varieties have different levels of sweetness. The estimated treatment ratings indicate that variety A is not as sweet as varieties B and C.

In order to check the assumption of group consistency, make the test of agreement. This requires values of B_1 for each judge as obtained from Table 1. Then, $B_1^e = 2.7669 + 2.2744 + 2.7669 = 7.8082$. The statistic,

$$\chi_4^2 = 4.6052\ (9.6199 - 7.8082) = 8.30.$$

This value of chi square is not significant at the level 0.05. It is large enough however to indicate that the assumption of group consistency should be critically examined for future experimentation with this panel.

If the experimenter had chosen a priori to admit the possibility of judge disagreement, he would have followed Steps $7(C)$ and $8(C)$. It has already been shown that $B_1^e = 7.8082$. Enter Table 2 under "3 equal groups," $n = 15$ and note that the observed value of B_1^e is significant at the 0.001 level. The conclusions would be unchanged in regard to the existence of treatment differences.

* Exact values of p_1, p_2, and p_3 are 0.4896, 0.4391, and 0.0713, respectively. Then $B_1 = 9.6200$ and $\chi_3^2 = 18.09$.

CONCLUSION AND SUMMARY

An experimental technique and analysis has been presented for paired comparisons which is simple and efficient in the sense that relatively small numbers of observations are required. The design has the advantage that it may be understood by the layman and the analysis requires no specialized statistical training. The method has enough flexibility to be adaptable to much sensory difference testing in that the effect of judges or groups may be controlled and a test of agreement performed.

Estimates of treatment ratings are provided which may be interpreted in a pairwise fashion and the logarithms of these ratings provide a scale of comparison for several treatments. This scale is a somewhat nebulous one but it should be noted that almost all scales used in subjective experimentation are somewhat arbitrary.

The procedure has been applied in numerous laboratory experiments which include the following at the Virginia Agricultural Experiment Station:

A. Testing for differences in cooked fresh pork and cured hams due to effects of diet.

B. The detection of benzene hexachloride and other insecticides in peanut butter.

C. The detection of color differences due to hormone spray treatments in a number of varieties of apples.

D. Testing for the effect of monosodium glutamate in apple sauce and dehydrated apples.

The authors are extending the new design and analysis to incomplete block designs with 3 treatments in a block and this will be the subject of a later report.

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Preservative Effect of Various Concentrations of Curing Salts in Comminuted Pork[a,b]

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The preservative effect of sodium chloride, sodium nitrate, and sodium nitrite in comminuted pork trimmings has been investigated. The pork was inoculated with known amounts of P. A. 3679 spores and incubated at 98.6° F. (37° C.) after which the preservative effect of the curing salts was determined by bacteriological and chemical analyses as well as by the appearance of the meat. The minimum amounts of the curing salts necessary both singly and in combination for the preservation of the meat are reported and discussed.

Processing schedules used by various manufacturers to assure satisfactory shelf life of canned meat products differ widely. However, regardless of the processing times followed in sterilizing pork luncheon meat products in 12-ounce oblong or 40-ounce round tins, no concrete evidence has ever been received from the field that any of these meats were understerilized *(16)*. A similar item merchandized in 3- and in 6-pound rectangular cans generally is considered to be understerilized since the heat believed necessary to destroy spores would cause excessive rendering and purging of the meat and result in an unpalatable product having low drained weight. While the perishable product does not have the pronounced canned or "overcooked" flavor associated with the item receiving a sterilizing treatment, the necessity of refrigeration restricts the use of luncheon meat packed in the larger containers.

Most spoilage of canned meat is considered to be caused by the group of bacteria known as putrefactive anaerobes. These organisms have heat resistant spores which can germinate and decompose meat under the anaerobic conditions provided within the sealed container. Generally, non-acid food, such as meat, is given a heat process calculated to destroy spores of *Clostridium botulinum*, an extremely toxigenic putrefactive anaerobe. Before a process of lower sterilizing value can be used, these and other viable putrefactive anaerobic spores must either be absent or be inhibited to a point where they will not produce toxin or cause spoilage. Earlier results from this laboratory *(2)* and elsewhere *(8)* have shown that fresh and cured pork trimmings contain light loads of anaerobic spores. Since these organisms have been found to persist in the product, it seemed important that the tolerance of such forms of life for the several chemical agents which comprise curing salt mixtures should be determined.

While the preservative value of curing salts has been recognized for some time, many conflicting reports regarding the concentrations which proved effective against putrefactive anaerobes are to be found in the literature. Some of the causes of these discrepancies were explained by Tanner and Evans *(17, 18, 19)* who observed that species and strains varied in susceptibility to the same salt and that similar organisms tolerated various levels of the same salt in different substrates. These workers also pointed out that another possible reason for contradictory results was the failure of many investigators to control salt concentrations by chemical analyses. The hydrogen ion concentration of the medium used to test the salt was shown to have an important influence upon the results by Tarr *(20)* who found that sodium nitrite was a more effective preservative in acid media. Lack of agreement of results might be due also to use of different spore loads in the curing salt tests. Jensen and Hess *(9)* and Stumbo, Gross, and Vinton *(15)* have shown that, as the number of spores added is increased, the curing agents are less effective in preventing spoilage.

There appears to be agreement among investigators on the value of sodium chloride in checking putrefactive anaerobic spoilage of canned meat, but there is difference of opinion regarding the preservative value of sodium nitrate and sodium nitrite. Ordinarily, commercial mixed cures contain relatively small amounts of sodium nitrate and sodium nitrite as compared with the amount of sodium chloride used. From a study made by Moulton and Lewis *(11)* they conclude that $NaNO_3$ and $NaNO_2$ exerted a specific inhibitory effect greater than that of an equal amount of NaCl. Yesair and Cameron *(21)* also concluded from their studies that nitrate and nitrite had a specific effect. On the other hand, Evans and Tanner *(3)* reported that putrefactive anaerobes would tolerate concentrations of a commercial mixture of NaCl, $NaNO_3$, and $NaNO_2$ up to the level where NaCl was effective when used alone. Gross, Vinton, and Martin *(6)* pointed out that the amount of $NaNO_3$ used in canned cured meat is too small to be of preservative value and that $NaNO_2$, while an effective preservative, is destroyed during the heat process.

It has been suggested by Jensen and Hess *(9)* that, in the presence of curing salts, putrefactive anaerobic spores may be destroyed at lower temperatures than in their absence. However, investigations by Yesair and Cameron *(21)* and by Stumbo, Gross, and Vinton *(15)* showed that the heat resistance values for putrefactive anaerobic spores were the same in cured as in uncured meat.

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The present study was undertaken to determine levels of the 3 curing salts which would be tolerated by putrefactive anaerobes in comminuted pork trimmings. Also, the effectiveness of a commercial curing salt mixture was investigated.

EXPERIMENTAL

Pork samples were obtained after they had been collected by a member of the laboratory staff of a commercial meat packing plant.[4] In securing samples, aseptic precautions were taken in transferring portions of the meat from each of the various trucks in the assembly room to a sterile metal container. Since the samples consisted of lean trimmings from the belly, jowl, backbone, loin end, lean butt, neckbone lean, blade meat, ham trims, and picnic trims and were selected from various loads, they represented meat from a considerable number of hogs. The composition of the pork trimmings was recorded and consisted of about 75% lean and 25% trimmable fat. The percent fat content was not determined by extraction but was that amount that could be trimmed by knife using ordinary care. Gross *(5)* indicated that at a level of 75 to 25 the trimmable fat and extracted fat values are approximately the same. After filling a 6-pound luncheon meat can with known quantities of each of the different types of trimmings in proper proportion, the can was hermetically sealed and immediately placed on a refrigerating coil at —25° F. (—31.7° C.). The contents were maintained in the frozen state during transit and until just prior to sampling in the college laboratory.

In order to prepare the meat for use, the can of frozen pork trimmings were placed in running tap water [60-65° F. (15.6-17.7° C.)] for ½ hour and, after opening the cans observing aseptic precautions, chunks of the still partially frozen meat were ground in a sterile meat grinder equipped with a 3/16 inch grinding plate. Following grinding the can contents were blended in a KitchenAid Model K4-B mixing bowl for 5 minutes at speed 10.

A standard spore suspension was made by transferring an inoculum of *Clostridium* species, No. 3679 National Canners Assoc. (P.A. 3679) to agar slants of Difco Linden thioglycollate medium modified by the addition of 1.5% agar and 0.1% soluble starch. The slants were incubated in the presence of purified "Seaford" nitrogen gas in accordance with the method described by Ogilvy, Burke, and Ayres *(12)* at 98.6° F. (37° C.) for 10 days. At that time a microscopic examination was made to determine purity and the presence of spores. The growth was washed from the slants with sterile physiological saline into a sterile container where it was heated at 176° F. (80° C.) for 20 minutes to destroy the vegetative cells. The spore suspension was held at 33° F. (0.6° C.) and, from time to time, counts were made using the "Most Probable Number" (M.P.N.) method.

Chemically pure curing salts were used, and in each instance a spore count was made to ascertain the degree of contamination.

The pork had a pH range of pH 5.8 ± 0.2. Addition of curing salts did not alter the pH of the fresh pork to any appreciable extent.

The preservative value of each of the salts was estimated in a preliminary experiment and, after the levels of the agent that would prevent germination completely had been determined, a retest was made using from 3 to 4 salt concentrations within that immediate range. Unsalted pork was used to check the effect of the added salt while uninoculated pork was tested to determine the extent of contamination of the meat. Each of from 5 to 7 replicate tubes were filled with approximately 15 g. of meat containing the appropriate level of salt and number of spores. A sterile calking gun fitted with an attachment for delivering the meat paste to the bottom of the test tube was used; care was taken to avoid entrapping excessive amounts of air. The tubes were held overnight at 33° F. (0.6° C.) and then heated in a water bath to an internal temperature of 176° F. (80° C.) for 20 minutes. After heating, one tube from each set was placed aside for the initial spore count and salt analysis; the remaining tubes were sealed immediately by the addition of a layer of sterile paraffin and then were incubated at 98.6° F. (37° C.) and examined at intervals of 1, 2, 4, 7, 14, and 28 days for visual signs of spoilage as indicated by color changes, gas production, and digestion of meat. At the end of 28 days one sample was withdrawn from each set for a spore count and salt analysis. In addition, this opened tube was judged for spoilage by odor and texture of the meat.

Spore counts were made by weighing 10 g. of meat, placing the meat in a Waring blendor and adding 250 ml. of physiological saline. After meat and saline were blended for 10 minutes, 5 replicate 10 ml., 1 ml., and 0.1 ml. quantities of the mixture were each added to tubes containing Difco Linden thioglycollate medium which had been modified by the addition of 0.1% soluble starch. These tubes then were heated at 176° F. (80° C.) for 20 minutes, sealed with a layer of sterile paraffin, and incubated for 10 days at 98.6° F. (37° C.). At the end of the incubation period positive tubes were determined by color change, meat digestion, cloudiness, and putrefactive odor; a microscopic examination was made of material from suspected and spoiled tubes. From the number of positive tubes in each dilution the most probable spore count was obtained by referring to M. P. N. tables.

Chloride was determined by precipitation with a known amount of standard $AgNO_3$ and titration of the excess Ag^+ with standard KSCN. The organic matter in the meat sample was destroyed by ashing in an oven or by wet oxidation with HNO_3 and $KMnO_4$. Results using the wet oxidation method proved to be both faster and more reproducible and, after the initial experiments, this latter procedure was used exclusively. A modification of the Schlössing-Wagner, or $FeCl_2$, method, recommended by Blake and Lewis *(1)* was used to determine nitrate. Sodium nitrite was determined by the method given by Morpeth *(10)* in which the nitrite ion is reacted with sulfanilic acid and α-naphthylamine to produce a red colored compound the intensity of which is an index of the nitrite present. Color intensity was measured by means of an Evelyn photoelectric colorimeter.

RESULTS

Sodium chloride. Various levels of sodium chloride were tested in attempts to determine the approximate minimum concentration of that salt required to prevent spoilage of inoculated and uninoculated pork trimmings. The amount of sodium chloride required in the meat containing no added spores varied from 2.0 to 4.3% between experiments. It was found that when the unsalted controls spoiled quickly, higher salt levels were necessary to keep the meat.

When a load of 175 spores of *Clostridium* spp. (P.A. 3679) per g. was added to pork, sodium chloride at a level of 4% by weight extended the storage life of the meat an additional 3 weeks beyond that of the raw unsalted meat or of pork containing only 2% added sodium chloride (Table 1). None of the inoculated samples containing 6% or 8% NaCl spoiled within the

TABLE 1

Spoilage of inoculated * pork containing different levels of NaCl (5 tubes)

Sodium chloride		Percent spoilage with incubation times at 98.6° F. (37° C.) of		
Added	Found by analysis	1 week	4 weeks	10 months
%	%			
0	0.1	100	....	....
2.0	2.1	100	....	....
4.0	4.1	0	100	....
6.0	6.1	0	0	0
8.0	8.2	0	0	0

* Analyzed to contain 175 P.A. 3679 spores per g.

[4] The authors are indebted to John Morrell and Company whose bacteriologists carefully collected and packed the lots of meat used in this study.

10-month holding period. In another trial, sodium chloride at a level of 5% was shown to delay the onset of spoilage by at least 6 months. Using these findings to establish the maximum and minimum limits of a bacteriostatic range, an attempt was made to determine more accurately the minimum inhibitory level. Concentrations of 3.5, 4.0, and 4.5% NaCl by weight were added to pork trimmings; also, samples containing no added salt and others containing neither salt nor organisms were used as controls. Each of two separate lots of pork were inoculated with 50 spores per g. of P.A. 3679. Results are shown in Table 2.

From these results it may be observed that the critical concentration of sodium chloride appears to be in the neighborhood of 4.0%. Although the minimum level of salt resulting in preservation was not always the same, undoubtedly the lack of reproducibility was due in part to the variability of spores normally present in the fresh pork as well as to those added. The number of spores present in the meat before inoculation was indicated by the fact that from 33 to 100% of the uninoculated tubes also immediately underwent spoilage anaerobically. A difference in susceptibility to sodium chloride among the various anaerobic spores normally present in meat would be expected.

Of some interest is the observation that, at a load of 175 P.A. 3679 spores per g., 4.1% NaCl did not prevent spoilage for 4 weeks (Table 1) but that when a smaller number of spores, 50 P.A. 3679 spores per g., were used (Table 2), lower levels of the agent were sufficient to preserve the meat.

Length of the incubation period also figured in the evaluation of the preservative properties of sodium chloride. As shown in the second trial in Table 2, none of the tubes containing 3.7% NaCl had spoiled during a 9-week incubation period but, at 16 weeks, all had spoiled.

A possible explanation for growth after long periods of incubation was offered by Halvorson *(7)* who suggested that hydroperoxides are likely to be present in media containing unsaturated fatty acids, that these peroxides can be decomposed by various types of enzymes, and that there may be a small amount of such enzyme activity in spores. Also, Halvorson postulates that, if there are a small number of spores, the peroxides may remain stable for relatively long periods of time but that upon standing they gradually disappear to a point where the spores can germinate. An interesting corollary to the above hypothesis would be that sodium chloride delays spore germination by interfering with enzyme elaboration.

Sodium nitrate. Since Tanner and Evans *(17)* indicated that the concentration of the curing agent (rather than the specific effect of the salt used) was the critical factor, the range, 3.5 to 4.5% sodium nitrate was selected for a preliminary trial. However, after a 1-week incubation period, signs of spoilage were evident in all tubes at every nitrate level. Typical anaerobic spoilage appeared first as a reddening of the meat. This was followed by darkening, foul odor, digestion and, with but few exceptions, gas production. Another type of spoilage in meat containing nitrate and arbitrarily considered as atypical, was characterized by change in color to gray-green, much gas production, no foul odor, and no extensive digestion. The pigment of the latter probably was similar to the oxidation pigments described by Greenwood, Urbain, Jensen, and Lewis *(4)* and caused by action of excess nitrite, oxygen, and microorganisms upon meat pigments. All spoilage of this type occurred within the first week of storage and did not increase upon further incubation (Table 3).

TABLE 3

Typical and atypical spoilage of inoculated [b] pork containing different levels of $NaNO_3$ (6 tubes)

Type spoilage	Sodium nitrate		Percent spoilage with incubation times in weeks at 98.6° F. (37° C.) of			
	Added	Found by analysis	1	2	4	12
	%	%				
Atypical	0	0	0	0	0	0
	3.5	3.5	17	17	17	17
	4.0	4.1	33	33	33	33
	4.5	4.7	33	33	33	33
Typical	0	0	100	....	....	....
	3.5	3.5	0	0	17	83
	4.0	4.1	0	0	0	0
	4.5	4.7	0	0	0	0

[b] Approximately 50 P.A. 3679 spores per g.

Some inhibition of anaerobic spoilage was noted in meat containing 3.5% $NaNO_3$ but, as was the case wherein NaCl was used at this level, the meat spoiled upon prolonged storage. Similarly, 4.1 and 4.7% $NaNO_3$ preserved meat for at least 12 weeks.

Since atypical spoilage appeared to be caused by an organism other than a putrefactive anaerobe, an

TABLE 2

Spoilage of inoculated [f] pork containing different levels of NaCl

Trial	Sodium chloride		No. of tubes	Percent spoilage with incubation times at 98.6° F. (37° C.) of				
	Added	Found by analysis		1 week	2 weeks	4 weeks	9 weeks	16 weeks
	%	%						
	0	0.1	6	100				
1	3.5	3.6	6	0	0	0	100	....
	4.0	4.0	6	0	0	0	0	....
	4.5	4.6	6	0	0	0	0	....
Uninoculated pork	0	0.1	6	33	67	67 [g]	100	....
	0	0.1	5	100				
2	3.5	3.7	5	0	0	0	0	100
	4.0	4.4	5	0	0	20	20	40
	4.5	4.6	5	0	0	0	0	0
Uninoculated pork	0	0.1	5	100	....	....	....	....

[f] Approximately 50 P.A. 3679 spores per g.
[g] 4 tubes spoiled; other 2 doubtful.

aerobic as well as an anaerobic spore count was made after the meat had incubated for 4 weeks. Atypically spoiled meat contained large numbers of spores capable of growing aerobically.

Also, nitrite analyses indicated approximately 2000 p.p.m. of $NaNO_2$ in atypically spoiled meat and none in sound or in anaerobically spoiled meat. These results indicate that atypical spoilage was due to an aerobic or facultative spore former which reduced nitrate to nitrite. These organisms were able to tolerate 10% $NaNO_3$. Quastel and Stephenson *(13)* have shown that nitrate can take the place of oxygen in the metabolism of some organisms.

In order to avoid the presence of spore forming aerobes, sterile cooked pork was used and a similar experiment was performed. Results corresponded to those of the first nitrate experiment if the atypical spoilage in the earlier trial is ignored (Table 4).

TABLE 4

Spoilage of inoculated [1] cooked pork containing different levels of $NaNO_3$ (5 tubes)

Sodium nitrate		Percent spoilage with incubation time in weeks at 98.6° F. (37° C.) of			
Added	Found by analysis	1	2	4	10
%	%				
0	0	100	....	....	....
2.9	3.1	100	....	....	....
3.4	3.8	0	100	....	....
3.9	4.1	0	0	0	0

[1] Approximately 50 P.A. 3679 spores per g.

Sodium nitrite. Preliminary experiments wherein widely different concentrations of sodium nitrite were used indicated preservation of pork at much lower levels than those which were effective with sodium chloride or sodium nitrate. Also, at high nitrite levels, rapid spoilage of the atypical kind observed in the first nitrate experiment occurred. A preservative effect was indicated at levels of from 0.04 to 0.08% $NaNO_2$ (Table 5) and longer storage life was observed for meat preserved with the higher nitrite concentrations. As shown by comparing the amount of nitrite added with that found by analysis, some destruction took place during the heat process. According to Greenwood, Urbain, Jensen, and Lewis *(4)*, nitrite destruction probably is caused by reaction with meat protein and is accelerated by elevated temperature. Chemical analyses made after 4 and 16-week storage periods indicate a continuing loss. Spoiled meat indicated absence of residual $NaNO_2$ and one sample that was still sound had only 0.0004% nitrite. Other workers, notably Ruyle and Tanner *(14)* have reported complete disappearance of nitrite in canned meats after several months of storage.

There was an appreciable reduction in the anaerobic spore count using the M.P.N. method, in preserved meat containing nitrite after it had incubated for 28 days (Table 6). However, since companion tubes to

TABLE 6

Anaerobic spore counts of meat containing different levels of $NaNO_2$

Sodium nitrite added by wt.	Spores per g. pork	
	At start of incubation	After 4 weeks incubation
%		
0.04	47	2
0.05	71	1
0.06	56	<1
0.07	38	1
0.08	115	1

those having a low spore count after the 4 week incubation period eventually spoiled, spore inhibition was not complete. The question might be raised whether growth of spoilage organisms may be due not to the development of tolerance for nitrite but rather to a gradual lowering of nitrite concentration until a non-toxic level is reached.

Mixed curing salts. Inoculated as well as uninoculated pork was used to test combinations of curing agents. The salts, sodium chloride, sodium nitrate, and sodium nitrite, were tested singly and in combination in concentrations used commercially (Table 7). In uninoculated meat, nitrite in combination with sodium chloride or nitrate appeared to extend storage life of the pork (Table 7A). From results of experiments in which inoculated meat was stored (Table 7B) it is noted that none of the salts or salt mixtures preserved pork upon prolonged incubation. The combination of all three of the salts was found to be the most effective but even this mixture could be tolerated by anaerobes when the meat was incubated at 98.6° F. (37° C.) for 19 weeks. In these experiments all inoculated control tubes spoiled within the first 24 hours of incubation. When used singly or with $NaNO_3$, neither NaCl or $NaNO_2$ had much preservative effect. On the other hand, the combination of chloride and nitrite had inhibitory action. In the experiment wherein $NaNO_2$ was introduced alone at the maximum tolerance permitted in meat products, spoilage was delayed to some extent and 3.5% NaCl when used alone also slightly prolonged storage life. When used by itself at the 0.125% level, $NaNO_3$ had no preservative effect.

TABLE 5

Spoilage of inoculated [1] pork containing different concentrations of $NaNO_2$

No. of tubes	Sodium nitrite				Percent spoilage with incubation time in weeks at 98.6° F. (37° C.) of			
	Added	Found by analysis after incubation time in weeks of						
		0	4	16	1	2	4	16
	%	%	%	%				
5	0	0	0	....	100	....	....	....
6	0.04	0.037[2]	0.0046	0	33	66	66	100
5	0.06	0.056	0.0058	0	20	20	20	100
5	0.08	0.073	0.0080	0.0004	0	0	0	20

[1] Approximately 50 P.A. 3679 spores per g.
[2] Percent $NaNO_2$ in sound samples of inoculated pork.

TABLE 7

Spoilage of pork containing NaCl, $NaNO_3$ and/or $NaNO_2$ at levels used in commerce

Trial	Spores per g. pork	No. of tubes	Sodium chloride		Sodium nitrate		Sodium nitrite		Percent spoilage with incubation times in weeks at 98.6° F. (37° C.) of					
			Added	Found by analysis	Added	Found by analysis	Added	Found by analysis	1	2	4	12	16	19
A. Uninoculated meat			%	%	%	%	%	%						
1	<1	5	0	0.1	0	0	0	0	20	20	20	20	20	20
		5	3.5	3.6	0.125	0.13	0.015	0.0083	0	0	0	0	0	0
2	<1	6	0	0.1	0	0	0	0	17	17	17	17	17	....
		6	3.5	3.6	0	0	0.017	0.0095	0	0	0	0	0	....
3	32	5	0	0.1	0	0	0	0	100	....	....	....	....	....
		5	3.5	3.6	0.125	0.13	0	0	100	....	....	....	....	....
4	52	5	0	0.1	0	0	0	0	80	100	....	....	....	....
		5	0	0.1	0.125	0.13	0.017	0.012	40	40	40	40	40	40
B. Inoculated [1] meat														
1		5	3.5	3.6	0.125	0.13	0.015	0.0083	0	0	0	0	20	100
2		5	3.5	3.4	0	0	0.017	0.012	0	0	0	100	....	....
		5	0	0.1	0	0	0.017	0.012	0	80	100	....	....	....
		5	3.5	3.5	0	0	0	0	0	100	....	....	....	....
3		4	3.5	3.7	0.125	0.13	0	0	50	100	....	....	....	....
4		4	0	0.1	0.125	0.12	0.017	0.011	100	....	....	....	....	....
5		5	0	0.1	0.125	0.12	0	0	100	....	....	....	....	....
Control		5	0	0.1	0	0	0	0	100	....	....	....	....	....

[1] Approximately 50 P.A. 3679 spores per g. added.

SUMMARY

An investigation was made of the effective preservative levels of the curing agents sodium chloride, sodium nitrate, and sodium nitrite in comminuted pork trimmings. The minimum concentration of each salt that would preserve pork inoculated with known loads of P.A. 3679 spores (approximately 50 per g.) was determined after incubation at 98.6° F. (37° C.) by bacteriological and chemical analyses as well as by appearance of the meat. Tubes of meat were heated to kill vegetative cells after which the pork trimmings were layered with melted paraffin to insure anaerobiosis. Anaerobic spore counts and curing salt analyses were made of representative samples at the time that the meat was placed in the incubator and again at the time of spoilage or at the end of 28 days (or more) of incubation. Varying degrees of bacteriostasis were shown by levels of 3.5 to 4.4% NaCl while no spoilage was observed at concentrations exceeding 4.4%. The anaerobic spores had about the same tolerance for $NaNO_3$ as for NaCl. Initial levels of $NaNO_2$ of 0.037% or more delayed meat spoilage. A gradual decrease in nitrite was noted during incubation and was accompanied by increased spoilage. Meat containing as little as 0.0004% nitrite apparently was sound while spoiled meat indicated absence of nitrite upon analysis. Inoculated meat containing the three curing agents tested individually or in combinations at levels permitted in commercial mixed cure showed that either $NaNO_2$ or NaCl delayed spoilage for from 1 to 4 weeks but that $NaNO_3$ had no inhibitory effect. The mixture of all three salts prevented spoilage of inoculated meat for 12 but not for 19 weeks.

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A Comparative Study of Tenderness Determination by Sensory Panel and by Shear Strength Measurements

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Tenderness determinations by a sensory panel and shear strength measurements by the Warner-Bratzler shear machine on broiled steaks from matched pairs of shortloins are reported and compared. The correlation coefficients obtained when the two methods are compared are discussed.

The food technologist has, in general, two methods for measuring quality attributes in foods: the sensory panel and instruments designed to measure certain physical and chemical properties of foods. For best results both methods must be especially adapted and critically evaluated for a particular type of investigation. In meat investigations tenderness is a consumer quality which has received much attention perhaps because it is considered the most important quality demanded by the consumer. For such studies tenderness determinations have been made using the panel techniques patterned after Cover *(2, 3)* and Deatherage and Reiman *(5)* or the Warner-Bratzler shear machine *(1)*. There have been no reports critically comparing these methods for determining tenderness. The brief report of Mackintosh, Hall, and Vail *(9)* summarized some data obtained over several years on panel tests, collagen determinations, and shear strength values. Although they indicate that panel testing gives reproducible values for tenderness, no data were given on the deviations in panel scores and shear strength values. Since quite a number of papers have been reported using one or another of these methods for measuring tenderness, it seems desirable to critically compare the results obtained by the methods using the same meat.

EXPERIMENTAL

The panel method of Deatherage and Reiman *(5)* which may be used so as to incorporate some features of Cover's paired eating method, and the Warner-Bratzler shear machine were used on broiled steaks from 32 pairs of matched shortloins from 32 commercially slaughtered steers. The tests were run in two groups of 16. One shortloin of each pair came from a side which had been processed by the so-called Tenderay method of James *(8)* while the other shortloin came from the corresponding side which had been kept as a control stored at approximately 35° F. (1.5° C.) until used. Thus, it was possible to compare the two methods for determinations on meat which had been aged by two different procedures.

Duplicate tests were run by the panel. For the shear machine, 1-inch cores were cut from the broiled steaks. The shear value in pounds represents the average of at least 4 measurements, two from each of two steaks adjacent to those used for the panel. For the shear machine in a few instances it was necessary to discard a reading which was obviously out of line and high due to a large piece of artery or connective tissue being included in the sample. No scores by the tasters were excluded.

The data obtained in the two series are shown in Tables 1 and 2. The grades noted are in accordance with the present grading system, even though originally the carcass grades were assigned prior to 1951. All tests were run 5 days post mortem.

TABLE 1

Tenderness and shear strength values on steaks from control and processed shortloins [a]

Animal No.	Tenderness by panel		Sheer strength in pounds		Preference by panel	
	Control	Processed	Control	Processed	Control	Processed
1	6.75	9.04	24.3	23.0	0	12
2	7.42	8.83	22.5	22.5	0.5	11.5
3	6.83	8.25	23.5	19.0	2.5	9.5
4	8.13	8.46	17.5	17.8	4	8
5	7.25	8.67	23.8	22.5	0	12
6	8.75	9.50	18.0	17.5	2	10
7	6.58	8.30	23.5	22.0	0.5	11.5
8	7.62	8.38	20.0	17.5	2	10
9	6.38	7.08	23.4	20.5	3	9
10	6.50	7.87	12.5	13.3	2	10
11	6.87	8.25	22.0	20.0	0.5	11.5
12	7.62	8.50	18.3	15.0	1	11
13	5.50	7.04	22.0	19.5	0	12
14	5.83	6.59	21.0	18.0	4	8
15	6.17	7.87	26.5	22.0	0	12
16	5.92	6.62	27.0	23.0	3	9
Total	110.12	129.25	345.8	313.1	25.5 [b]	166.5 [b]
Average	6.88	8.08	21.6	19.6	1.6	10.4
Standard error of mean	0.23	0.21	0.92	0.74	.....	.....

[a] Steers, approximately 1½ years old; average carcass weight 502 lb.; 12 grading Choice and 4 Good.
[b] Chi square is 103.5; at 1% level chi-square is 6.6.

TABLE 2

Tenderness and shear strength values from control and processed shortloins [c]

Animal No.	Tenderness by panel		Sheer strength in pounds		Preference by panel	
	Control	Processed	Control	Processed	Control	Processed
17	7.17	8.71	19.6	18.8	2	10
18	7.46	8.34	18.6	20.0	2	10
19	7.38	8.21	23.9	20.8	2.5	9.5
20	7.75	8.79	24.1	17.4	2.5	9.5
21	7.75	8.29	22.0	20.3	4	8
22	7.67	8.29	24.4	18.0	3.5	8.5
23	7.67	8.79	17.8	14.0	1	11
24	7.63	8.25	17.6	16.4	1.5	10.5
25	7.46	8.84	24.4	20.5	2	10
26	7.38	7.54	23.1	26.0	6.5	5.5
27	7.71	9.08	19.4	17.5	2.5	9.5
28	6.42	8.00	22.8	19.1	2	10
29	7.84	8.58	20.0	18.6	2	10
30	7.50	8.54	25.8	24.1	3	9
31	8.88	9.42	16.0	16.1	2	10
32	7.04	8.83	19.1	17.8	2.5	9.5
Total	120.71	136.50	338 6	305.4	41.5 [d]	150.5 [d]
Average	7.54	8.53	21.2	19.1	2.6	9.4
Standard error of mean	0.10	0.11	0.62	0.72	.....	.....

[c] Steers, average age 1½-2years, average carcass weight 570 lb.; 2 grading Choice, 7 low Choice, and 7 Good.
[d] Chi square is 61.9.

Before discussing the data in Tables 1 and 2 it might be well to point out that a study of the reproducibility of taste test data has shown that the standard deviation for the average panel score for 6 judges is approximately 0.46-0.73 depending on the tenderness range

(5, 7). For the duplicate tests, the tenderness scores which are noted in Tables 1 and 2, the standard and probable deviation ranges are 0.33-0.52 and 0.22-0.35, respectively. Similar computations to indicate reproducibility for the shear values in these tables indicate that the standard and probable deviations for these data are 2.0-2.2 and 1.3-1.5 pounds with several values excluded for the reasons given above. For reasons of brevity, the individual shear values which were averaged to get each entry in the above tables are not given. The deviations indicating degree of reproducibility for each datum in these tables were not incorporated in the computation of the standard error of mean in Table 1 and 2. If these calculations are carried out, the results do not greatly affect the over-all picture particularly concerning tenderness by panel.

In both groups of tests the taste panel shows definite differences in tenderness between the control and processed steaks. The processed meat as a group is more tender by tenderness score and by preference score even though the meat in general was quite acceptably tender before processing.

The shear values were less for the processed meat in each group, and the differences in mean shear values in each table appear to be significant even though the differences are not as great as that from the taste panel data. Therefore, some relation between tenderness and shear value may exist.

When shear values and panel scores are more closely compared, it is at once apparent that the relation between the two is not as close as would be desirable. A correlation coefficient of —0.369 is obtained when shear strengths and panel scores are compared for the 32 control sides. For the processed meat a similar comparison gives a correlation coefficient of —0.242. These values are not very significant since for 30 degrees of freedom the values for the correlation coefficient at the 1% and 5% levels should be —0.449 and —0.349.

In an effort to determine if increases in tenderness as determined by the panel were related to decreases in shear strength as determined by the machine, these two factors were compared in the 32 carcasses. A correlation coefficient of 0.173 indicates essentially no direct relation between these two quantities.

Tenderness values by the panel on meat 2 to 3 days post mortem usually correlates well with tenderness values at 15 days post mortem. Similar close relation between control and processed beef has been observed. Correlation coefficients of 0.80-0.90 are usually obtained with the value depending upon the tenderness level of the meat under study. In these series of tests the tenderness values of the control and those of the corresponding processed sides had a correlation coefficient of 0.792. Similarly, the shear strengths of the control and the processed meat had a correlation coefficient of 0.783. Therefore, it is clear that shear strength by the Warner-Bratzler machine and tenderness measurements by the panel both measure some property of meat in a fairly reproducible manner. Furthermore, these properties change with post mortem age of the meat and each may be based fundamentally on more than one variable. Indeed, there is ample evidence that this is true for tenderness as measured by the panel *(4)*. However, in view of the poor correlation between taste panel and shear machine, it must be presumed that shear strength and tenderness are not the same property of meat. It may be that shear strength measures a variable which is related to tenderness. Even so, it appears that this variable is not the dominant one related to tenderness of meat. Consequently, the synomymous use of the terms shear strength (as determined by the Warner-Bratzler machine) and tenderness of meat should be avoided.

There are a number of statements in the literature indicating a higher correlation than is reported here between panel score and shear values. It is not possible to determine why this is so since complete detailed data for study is often not published but are usually summarized *(9)*. One reason may be that higher values might be expected if the variations in meat were greater. Evidence that this might be the case is seen in the higher correlation in the control meat than in the processed meat. In this study there was not a wide spread in tenderness level, but the control samples had a wider range of tenderness values. For research, it is necessary to have a reproducible and discriminating test for tenderness rather than one sensitive only to large differences on tenderness.

When preference judgments patterned after Cover's method are examined, it is clear that there are real differences in tenderness between the control and the processed meat of each group. The preference scores give essentially the same result as the tenderness level scores. This confirms the previous report of Deatherage and Reiman which critically compared preference scores and tenderness scores.

In the brief report Mackintosh, Hall, and Vail *(9)* reported almost perfect correlation (-0.986 ± 0.003) between panel scores and tenderness on meat from a selected group of 45 steers and in another grouping of 61, presumably containing some of the 45, they give a coefficient of -0.60 ± 0.6. In a detailed and excellent report on beef quality from steers fed low phosphorus and high calcium rations, these same authors *(6)* give panel scores and shear values on both fresh and aged meat from 22 different steers. These data have been pooled and subjected to study in the same manner as described in this paper. The same conclusions as given above were reached. On the fresh meat, there appears to be some correlation between tenderness and shear values (-0.541) on fresh samples but none (-0.159) on aged. The shear values for fresh meat did not correlate with the shear values for the aged meat (0.292). However, the panel score on fresh meat showed good agreement with a correlation coefficient of 0.724. For 20 degrees of freedom 1% and 5% levels of significance are 0.537 and 0.423. If 2 of the 22 shear values on fresh meat are discarded as being much too high and in error, the 0.292 becomes 0.666 for shear value agreement, but the -0.541 becomes only -0.463 for panel-machine agreement. It should be noted also that on the basis of a 7-point scoring system instead of 10 as used in this study, the mean tenderness was 5.63 ± 0.10 for the fresh and 5.82 ± 0.10 for the "ripe." The mean shear value for the "ripe" meat was 13.7 ± 0.5 and for the fresh meat it was 15.3 ± 0.6. The latter value would

be 14.7 ± 0.5 if the two shear values noted above are not considered.

It would be desirable to know precisely what tissues or substances in meat are responsible for shear strength values as this might help understand the nature of tenderness in meat. However, for fundamental investigations on tenderness as a consumer quality attribute in meat, the sensory panel appears to be the preferred method for guiding research. Although so-called objective methods may have some advantages, these are of secondary importance until an objective test or machine is developed which gives results which correlate very closely to tenderness as judged by the consumer or by sensory panels. It may be that further study of the shear machine could lead to a device which agrees more closely with tenderness.

SUMMARY AND CONCLUSIONS

A comparative study has been made of tenderness determinations by a sensory panel and shear strength measurements by the Warner-Bratzler shear machine on broiled steaks from matched pairs of shortloins from 32 steers. One side from each steer was aged at elevated temperature while the other was kept as a control. Although shear strengths appear to measure fairly satisfactorily a property of meat, these values are not closely related to tenderness of broiled steaks as determined by a competent sensory panel.

Further critical study of the shear measurements may give useful fundamental information on meat.

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Surface Formation and Shear as Factors Affecting the Beating Power of Egg White*

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The effects of shear forces and of surface formation on the beating power of eggs were studied by means of an electric mixer and a Bull surface denaturation device. The results indicate that shear forces play a dominant role in the destruction of beating power during atomization. Surface may also play a role, especially if the amount of surface formed and the rate of formation are high.

Over the years the frozen and dried egg industry has become fully aware of the fact that egg white is extremely sensitive to heat and other physical agents. Relatively small changes in the processing and handling of egg products are known to adversely affect functional properties. One of the most significant examples of this problem is the almost complete loss in the beating power of egg white when it is spray dried using conventional pressure nozzles and centrifugal atomizers. The damage occurs under conditions of temperature and time well below those known to cause heat damage. This is a serious problem because without a solution to it a drying technique possessing many desirable characteristics like rapid drying, relatively low drying temperatures, high products output per drying unit, low labor cost, etc. can not be applied in the commercial production of this commodity.

Developmental work of the past 20 years has produced certain improvements in the drying of albumen. For example, it has been found that prolonged fermentation *(5)* and acidification prior to drying *(3)* aid in producing a spray dried albumen with good whipping power. However, while products made by the application of these techniques are being used successfully in candies, meringues, and certain cake mixes, they are not very satisfactory for angel cake.

In the course of other studies devoted to the basic aspects of this problem, it has become increasingly clear that shear forces and surface formation effects were involved in the loss of beating power. The work of Slosberg *(8)*, Forsythe and Bergquist *(4)* and MacDonnell, Hanson, Silva, Lineweaver, and Feeney *(6)* clearly shows that the shear forces encountered in homogenization and continuous supercentrifuging will adversely affect beating power. It is well known that the conventional atomizing devices (pressure nozzles and centrifugal atomizers) used in spray drying exert considerable homogenizing (shearing) action on the liquids passing through them *(7)*. In addition, atomization also creates enormous surfaces which may be the cause of some of the loss in beating power.

In order to throw light on this problem, experiments were carried out to study the effect of surface formation on beating power. Certain additional studies were also made on the effect of shear on this property of egg white.

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EXPERIMENTAL

The effects of surface formation were studied by means of an electric mixer (Hobart KitchenAid, Model 4, with wire whip), and a Bull surface denaturation device *(2)*. The latter consists of a motor-driven porcelain postage stamp moistener (Figure 1). The cylinder is 7.3 cm. in diameter and 5.5 cm. long. Allowing for contact between the sides as well as the face of the cylinder and the liquid, the total surface area formed per revolution is 132 sq. cm. The cylinder is rotated by a Cenco No. 18805 variable-speed stirrer assembly.

Beating power was determined by using the meringue test described by Slosberg, Hanson, Stewart, and Lowe *(9)*.

The egg white used for these trials was the commercial milled [e] and frozen product. To assure uniformity of raw material for the experiments, a sufficient supply for all experiments was obtained from one lot of albumen, all of which came from one well mixed batch. When needed, fermentations were carried out by inoculating the egg white with a culture of a special strain of *Aerobacter aerogenes* and incubating at 34° C. until the sample was free of reducing sugar.

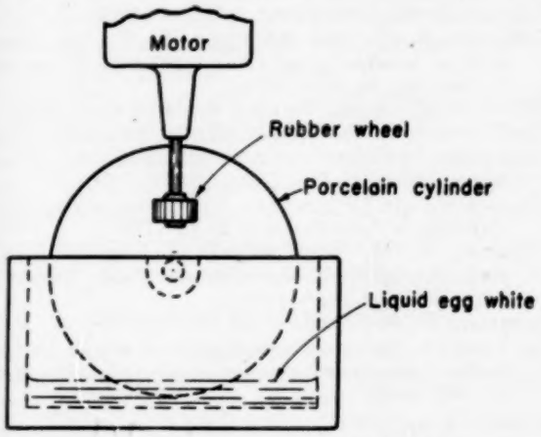

Figure 1. Surface formation device.

RESULTS

A number of samples of spray-dried albumen were obtained from commercial sources and were examined for appearance and particle size. As expected, the particles were spherical in shape; they showed some tendency to agglomerate. They had an average diameter [d] of 44.0 microns. Separate studies showed that the diameter of the atomized droplets decreased about 25% during drying; this would make the average of atomized liquid approximately 1000 sq. cm. per ml. This information served as a basis of comparison for the experimental samples.

The first studies relative to the effect of surface formation were made using the KitchenAid electric mixer. In these trials the egg white was beaten at several different rates for varying intervals in order to provide a wide range of foam density. The foams were then broken down by allowing them to stand (with occasional stirring), and the resulting liquid was then tested for beating power.

Table 1 shows some typical data obtained in these studies. It is clearly evident that the beating power of

[e] The process consists of passing egg white through a high-speed chopper (Tranin mill). The action of the special blades in the rotor is such as to thoroughly break down the thick white and incorporate it into the thin. The product emerging from the chopper is homogeneous and free-flowing.

[d] The term diameter as used here is that of a single particle with the same ratio as the total sum of particles measured.

TABLE 1

Effect of beating egg white on subsequent beating power of disintegrated foam

Speed of mixer	Time of initial beating	Trial No. 1 [e] Specific volume of foam	Trial No. 1 [e] Beating rate of disintegrated foam	Trial No. 2 [e] Specific volume of foam	Trial No. 2 [e] Beating rate of disintegrated foam	Trial No. 3 [f] Specific volume of foam	Trial No. 3 [f] Beating rate of disintegrated foam
	seconds	*ml. per g.*	*ml. per g. per minute*	*ml. per g.*	*ml. per g. per minute*	*ml. per g.*	*ml. per g. per minute*
Control	.....	.....	4.25	.....	4.10	.....	3.52
1 (low)	120	5.32	4.30	5.52	4.48	.....	.....
	180	5.65	4.36	5.91	4.30	.....	.....
	240	5.99	4.36	6.05	4.05	.....	.....
2 (medium)	30	7.80	4.42	8.33	4.42	5.98	3.38
	60	9.10	4.36	8.85	4.42	6.25	3.56
	90	9.44	4.10	9.00	3.56	6.58	3.10
3 (high)	20	9.51	4.25	9.10	4.30	7.15	3.52
	40	10.20	3.86	9.90	3.42	9.90	3.04
	60	10.52	3.14	10.52	2.95	11.75	2.36

[e] Commercial frozen egg white, unfermented.
[f] Commercial frozen egg white, Aerobacter fermented.

the treated albumen was not affected very much until it had first been formed into foams of very high specific volume (low density). For example, at a speed of 3 for 60 seconds where the beating rate of the treated albumen was definitely affected, the mean bubble diameter of the foam was 10 microns. This corresponded to 5,200 sq. cm. per g. which is several times that found in the commercially atomized particles.

More quantitative studies of the effect of surface formation and ones in which shear was further minimized were made using the Bull surface forming device. With this apparatus surface formation, both with respect to the absolute amount as well as rate of formation, can be very accurately controlled. Figure 2 shows some of the data obtained in these trials. These results con-

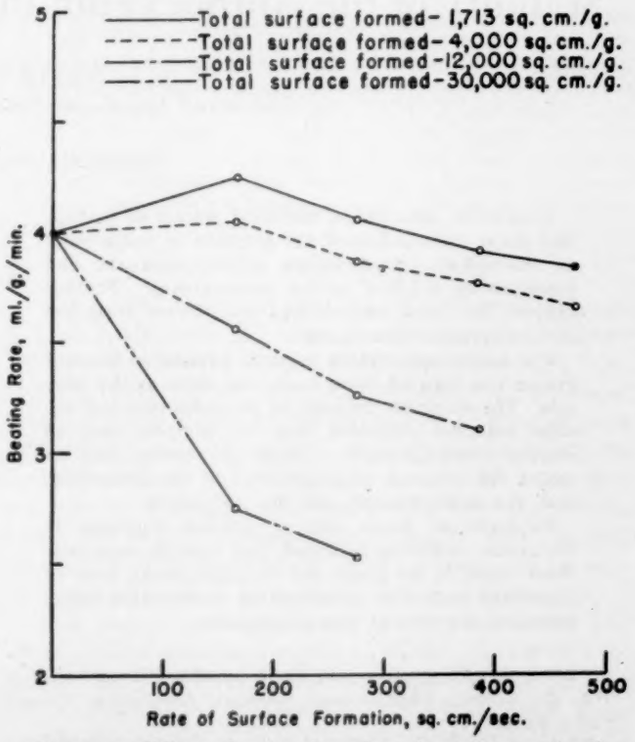

Figure 2. Effect of rate of surface formation on beating rate of egg white.

firm and extend those given above. Not only was the beating rate affected by the amount of surface formed, but the rate of surface formation also had a decided influence on this property. It should be noted, however, that the rate of surface formation during atomization is extremely high compared to that encountered here. Unfortunately it was not possible to obtain data at higher rates of surface formation, using the surface formation device since excessive foaming interfered with the operation of the unit.

Trials were then made to provide some information on the effect of the solid-to-liquid shear force encountered in the surface formation experiments on beating power. To do this a test tube of 2.5 cm. diameter was rotated in a sample of egg white at the rate of 223 r.p.m. for 224 minutes. This gave a peripheral speed equivalent to the surface formation device cylinder rotating at 167 r.p.m. and a solid-to-liquid shear area of 30,000 sq. cm. per g. of liquid. This treatment had no effect whatsoever on the beating power of the albumen. This shows that the shear forces encountered even in the most severe surface formation trials were without effect on the egg white. Taken together the data clearly demonstrate that surface formation per se can be destructive to egg white properties. However, it seems unlikely that, with the amount of surface formed during atomization, surface effects are the primary cause of the damage.

The work of Slosberg *(8)*, MacDonnell, et al. *(6)* and Forsythe and Bergquist *(4)* makes evident the importance of shear force in the destruction of beating power in egg white. As stated before, the pressure nozzles and centrifugal atomizers commonly used in spray drying egg products are known to have large homogenizing (shear) effects. In the face of this and the new information presented here, we suggest that shear forces play the dominant role in the destruction of beating power during atomization. Surface formation may also play a role, especially if the amount of surface formed and rate of formation are high.

Attempts to reduce shear forces and rate of surface formation in spray drying egg white will be described in another article *(1)*.

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Stability of the Anthocyanin Pigments in Concord Grape Juice[a,b]

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Qualitative tests on the methanol extract of lyophilised grape skins indicated the presence in grape juice of chlorophyll, water soluble yellow pigments, and carotenes in addition to the anthocyanins. Further support for these observations was drawn from the chromatographic techniques.

The major anthocyanin pigment present in Concord grapes was isolated from fresh ripe skins as the chloride. The empirical formula of the substance and the color reactions indicated that the pigment may be oenidin 3-monoglucoside. Paper chromatography revealed the presence in grape juice of the anthocyanidin, the monoglycoside and the diglycoside.

Exposure of juices and of purified pigments to ultraviolet radiation indicated that tannins, especially those found in the grape and in grape stems, have an important protective action on the anthocyanin which decreases the rate of loss of pigment.

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[b] Journal Paper No. J-2002 of the Iowa Agricultural Experiment Station, Ames, Iowa Project No. 1051.

The study of the grape anthocyanins over the past half a century has shown that these pigments are as a rule the glucosides of delphinidin and its methyl ethers. Willstätter and Zöllinger *(18)* showed that the pigment in the dark blue European grape *Vitis vinifera*, consisted largely of the monoglucoside, oenin, isolated as oenin chloride. From his investigations on American grapes, Anderson *(1)* indicated that the colored substance in three varieties of these grapes, namely *Vitis aestivalis* Michx. (Norton), *V. labrusca* (Concord), and *V. riparia Michx.*, consisted of an anthocyanin which on hydrolysis yielded a monomethyl ether of delphinidin, or possibly a mixture of delphinidin dimethyl ether and delphinidin in equimolecular proportions. It was also stated that there was evidence for the presence of some diglucoside and some free anthocyanidin in these grape varieties. Levy, Posternack, and Robinson *(10)* found in the fogarina grape a small amount of delphinidin and its 3′-methyl ether in addition to oenidin. Cornforth *(6)* isolated from *Vitis hypoglauca*, an Aus-

tralian wild grape, an anthocyanin which was shown to be largely oenin (3′-5′ dimethyl 3-monoglucoside of delphinidin). Browne *(5)* isolated the anthocyanin from Muscadine grapes and showed that it consisted of 3′, 5′-diglucoside of petunidin and no evidence of a monoglucosidal anthocyanin was found. Mackinney *(11)* stated that very low concentrations of chlorophyll were found by spectrophotometric methods in Sultanina grapes and raisins.

Nebesky, Esselen, McConnell, and Fellers *(12)*, working with purified anthocyanin pigment solutions from strawberries and currants observed that high storage temperatures and the presence of oxygen accelerated color deterioration with both pigments, while a low storage temperature preserved the color. The strawberry pigment solutions, unlike the currant pigment solutions, developed a faint precipitate after storage for a week. This precipitate increased on further storage. Sugar had little effect on the stability of the color in the pigment solutions.

The present investigation was made to determine the nature of the water-soluble pigments in Concord grape juice and to demonstrate their stability under the influence of the conditions of storage. In addition, investigations were made of the nature of the substances present in grape juice responsible for the protective influence on the pigment solutions during storage.

EXPERIMENTAL PROCEDURE

For the qualitative identification of the water-soluble and insoluble pigments present in grapes, the method suggested by Bancroft and Rutzler *(2)* was employed.

The method used for the isolation of the major anthocyanin pigment from Concord grapes was essentially the one followed by Anderson *(1)* in his investigations with Concord grapes. To 3 g. of the crude glycoside dissolved in 20 ml. of methanol, was added 25 ml. of ethanol containing 7 ml. of 21% hydrochloric acid in absolute alcohol. The clear filtrate was allowed to stand for 3 days at room temperature in a loosely covered dish. Amorphous masses of the pigment precipitated out and the substance could not be obtained in a crystalline state even though the conditions for crystallization were varied.

For the separation of the anthocyanidin, its mono- and diglycosides, the method employed was essentially the one suggested by Willstätter and Everest *(17)*.

The anthocyanin concentrations of the pigment solutions were determined by utilizing the property that the optical densities of the clear pigment solutions of pH 1.0 or less observed at a wave length of 515 mμ, bear a linear relationship to the pigment concentrations over a limited range. For the estimation of tannins, the method of Hartmann *(8)* was adopted. The method of French, Knapp, and Pazur *(7)* was used to identify the sugar present in the anthocyanin in glycosidic form. For this purpose the pigment solution was hydrolyzed by boiling with dilute HCl for 15 minutes and the anthocyanidin was removed from the boiled acid solution by repeated extraction with isoamyl alcohol. The acid solution containing the sugars was adjusted to pH 3.4 with dilute ammonia (10%) and then evaporated to dryness in a vacuum oven at a temperature of 95° F. (35° C.) under a pressure of 1 mm. The dried extract was dissolved in distilled water, and the sugar present was identified by use of paper chromatography.

Pigment solutions were exposed to ultraviolet radiation which apparently accelerated destruction of the pigment, and made short period studies feasible. A General Electric 15 watt ultraviolet lamp 17.5 inches in length was fitted into a fluorescent fixture and used in studies of the effects of exposure to ultraviolet radiation. The lamp emitted radiations mainly in the region of 2537 A. Grape juice samples of pH 2.4, 2.9, 3.4, 3,9, and 4.4 were exposed to ultraviolet radiation for a period of 48 hours. Similarly anthocyanin chloride solutions adjusted to the same pH values were exposed to ultraviolet radiation.

Tannins affect the stability of anthocyanins exposed to ultraviolet radiation *(15)*. To demonstrate this effect the tannins were separated from the grape juice by the method employed by Johnson, Foreman, and Mayer *(9)*, with a few modifications. To a mixture of 100 ml. of grape juice and 25 ml. of 95% alcohol, saturated neutral lead acetate was added with stirring until no further precipitation occurred. The mixture was centrifuged after allowing the contents to remain at 104° F. (40° C.) over night. Dilute sulphuric acid was added to the clear supernatant liquid and the mixture was left at 104° F. over night to precipitate soluble lead salts as insoluble lead sulphate. The solution containing the insoluble lead sulphate was filtered and the clear filtrate was adjusted to pH 3.4 with 10% ammonia. The filtrate was concentrated in a vacuum oven at 104° F. under a reduced pressure of 1 mm. until the volume was reduced to less than 50 ml. The concentrated solution was then made up to 50 ml. with a McIlvaine's buffer solution of pH 3.4. This extract served as tannin-free juice for ultraviolet exposure studies.

The insoluble lead precipitate was decomposed by adding a solution of 1 *N*. H_2SO_4 until no further precipitation of lead as sulphate occurred. The solution was cooled over night and then centrifuged for 10 minutes at 1500 r.p.m. The clear filtrate containing tannins and colored substances was adjusted to a pH of 3.4 with 10% ammonia and then concentrated in a vacuum oven at 104° F. (40° C.) under a reduced pressure of 1 mm. till the volume was reduced to less than 50 ml. It was then made up to 50 ml. with a buffer solution of pH 3.4. This extract was added to the tannin-free juice in ultraviolet exposure studies.

For the preparation of the extract containing tannins and other polyphenolic substances from grape stems for the ultraviolet exposure studies, the stems from grapes stored at 0° ± 1° F. (—17.8° C.) were dried at 104° F. (40° C.) under a pressure of 1 mm. The dried stems were mixed with 10 g. of sea sand and crushed in a mortar. The crushed mass was extracted by boiling with 500 ml. of water. The sea sand and the water-insoluble stem material were separated by filtration of the boiled mixture through a Buchner funnel under suction. Saturated neutral lead acetate was added to the clear filtrate to precipitate the tannins and polyphenolic substances as insoluble lead salts. The remainder of the procedure was identical with the one followed in the extraction of tannin-free juice. The extract was used for ultraviolet exposure studies. The different components present in grape juice, that is, sugar (18%), tannin (0.2%), pectin (0.15%), tartaric (0.4%) and malic acids (0.5%) were added to 20 ml. of purified pigment solutions (pH 3.4) at the levels present in the juice. Pure tannic acid was substituted for tannin. The mixed solutions of pigment and the other juice components were exposed to ultraviolet radiation.

RESULTS AND DISCUSSION

The yields of anthocyanin chloride obtained through use of Anderson's *(1)* method were very low. About 10 g. of crude anthocyanin chloride was obtained from 4000 g. of fresh grape skins and the yields were reduced when crystallization of the anthocyanin chloride was attempted. All attempts to crystallize the substance failed, yielding in every case brownish red, tiny, amorphous granules in clusters. To free them from ether soluble impurities, these granules were washed thoroughly with anhydrous ether. The resulting substance did not show any trace of metals or nitrogen.

The percentage composition of the individual elements was determined to be: C, 52%; H, 4.66%; Cl, 6.5%; H_2O, 7.4%. Calculated values were: 52.2%; H, 4.73%; Cl, 6.71%; and H_2O, 7.84%. From these results the empirical formula assigned was $C_{23}H_{25}O_{12}Cl$ and this agreed closely with the calculated values.

The R_f value of the pigment in dilute HCl solution was determined to be 0.21 using the nonaqueous layer of the butanol, acetic acid, and water mixture (4:1:5) and Whatman No. 4 filter paper at 70° ± 0.5° F. (21.1° C.) for the chromatography.

The sugar present in the pigment molecule in the glycosidic form was identified by paper chromatography after hydrolyzing the pigment to the aglycone and sugar. The apparent R_f value of the sugar present in the pigment after 3 passes exactly equalled that of glucose (0.63) under identical conditions, confirming the presence of glucose in glycosidic form.

The qualitative tests for the various pigments present in 2.5 g. of lyophilized grape skins performed according to the method suggested by Bancroft and Rutzler *(2)* indicated the presence of chlorophyll, water soluble yellow pigments, and carotenes in addition to the free anthocyanidin and its glycosides.

When chromatographic separation of the juice samples was employed using alumina as the adsorbent, the colored substance was adsorbed at the top of the column and no separate bands could be noticed. Similar results were obtained when the adsorption capacity of alumina was reduced by mixing with Dicalite (1:2 by volume). The blue band formed on the alumina column was eluted with dilute HCl, and this acid extract showed its absorption maximum at 515 mμ. When the petroleum ether and benzene (9:1 by volume) extract of the lyophilized grape skins was chromatographed using sucrose as the adsorbent, a faint green band appeared in the middle of the column, while a yellow band was observed by partitioning the same extract with magnesia as adsorbent. The observations indicated the possible presence of chlorophyll, carotene, and water-soluble yellow pigments *(16)* in grape skins. Further work to identify the fat-soluble pigments was not pursued as the pigments were apparently not extracted into the processed juices.

By using a few drops of an extract obtained from lyophilized grape skins by methanol containing 1% HCl, chromatographic separation was attempted employing Whatman No. 4 filter paper strips. The nonaqueous layer of the mixture of butanol, acetic acid, and water (4:1:5 by volume) was used as the flowing solvent and the separation was affected at a constant temperature of 70° ± 0.5° F. (21.1° C.). A separation was affected into 2 distinct red bands near the bottom and a hazy one near the top of the paper. The R_f values of the lower band which turned purple on drying and of the upper band which remained red, were found to be 0.11 and 0.19, respectively. It was observed that the red band at the top with an R_f value of 0.56 was destroyed in the course of approximately 24 hours while the other 2 bands were quite stable. Purified pigment solutions prepared by the method suggested by Reynolds, Robinson, and Scott-Moncrieff *(13)* and free from fat-soluble pigments did not indicate the presence of the greenish yellow band observed when the methanol —HCl extract of skins was separated on the paper. On exposure to HCl fumes, both of the bands turned red. Exposure to ammonia fumes changed the colors to bluish green, with the red band regaining its original color in the course of a few hours. On spraying dilute ferric chloride solution on the paper after separation, the color of the lower band appeared purple and the upper one reddish violet.

When the dilute acid (2%) extract of the purified pigment solution was hydrolyzed by boiling for 15 minutes and the solution after hydrolysis chromatograped using butanol, acetic acid, and water, the lower bands were not noted, and a hazy trail of red pigment with an R_f value near 0.56 appeared on the chromatogram indicating that the purple and red bands (with R_f values 0.11 and 0.19) were hydrolyzed to the red band with an R_f value (0.56), which disappeared in the course of 24 hours.

The bands of the red and purple color from many paper chromatograms were cut and the separate strips were dipped in dilute HCl and the absorption values of the acid solutions were determined with the spectrophotometer (Figure 1). The shapes of both of the

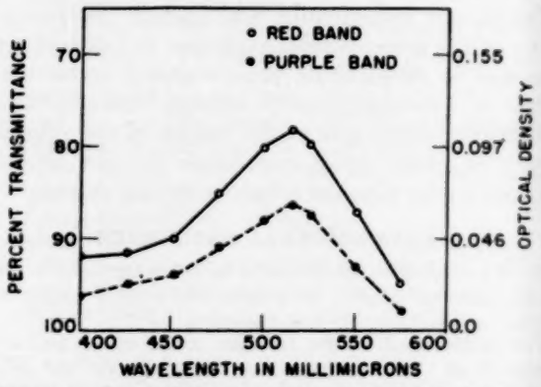

Figure 1. Transmittance values of acid solutions of red and purple bands after separation of mixed pigment extracts by paper chromatography.

absorption curves are similar with an absorption maximum at 515 mμ. The curves also indicate that in the pigment extracts, the pigment component responsible for the red band predominates over the pigment component causing the purple band.

Further attempts to identify the two bands were made by separating the pigment extract into the diglycoside and monoglycoside by repeated extractions with isoamyl alcohol. The R_f values of the di- and mono-glycosides were determined individually under identical conditions. It was noted that the R_f value of the lower purple band equalled that of the diglycoside while that of the monoglycoside corresponded with the upper red band. These findings suggested that the upper band was the monoglycoside and the lower band the diglycoside of the anthocyanidin while the band at the top represented the sugar-free anthocyanidin. Further support for these assumptions was gained from the purple color of the lower band and the red color of the upper band, in agreement with the observations of Robinson and Robinson *(14)* that, in general, the diglycosides tend to be bluer than the corresponding monoglycosides.

Using fresh grape juice acidified with dilute HCl, the same number of bands were noticed in the middle with a hazy band at the top. The R_f values of the purple and red bands coincided with those obtained with purified

pigment solutions. The bands obtained with the juice were, however, slightly broader than the ones obtained with purified pigment solutions, probably due to the effects of the other juice components. The resolution in the case of grape juice was improved by allowing the flowing solvent to run through the column a second time after the column was dried. In this case only apparent R_f values could be determined. Substituting Whatman No. 1 for Whatman No. 4 filter papers appeared to increase the R_f values (0.12 and 0.21). If water extracts of the pigments were used instead of the acid extracts, some hazy bands appeared at the top of the paper probably due to the neutral form of the color base.

The purified di- and monoglycosides separated from each other were concentrated and convenient volumes of the concentrated solutions were added to 15 ml. of buffer solutions with pH values of 1.0, 1.5, 2.0, 2.5, 3.0, 3.4, 4.0, 7.0, and 8.0. The absorption values throughout the entire visible range were determined at the various pH values with the spectrophotometer. The effects of pH on all three pigment components were similar and, as a result, only the curves for the monoglycoside are presented in Figure 2. Figure 3 indicates that at

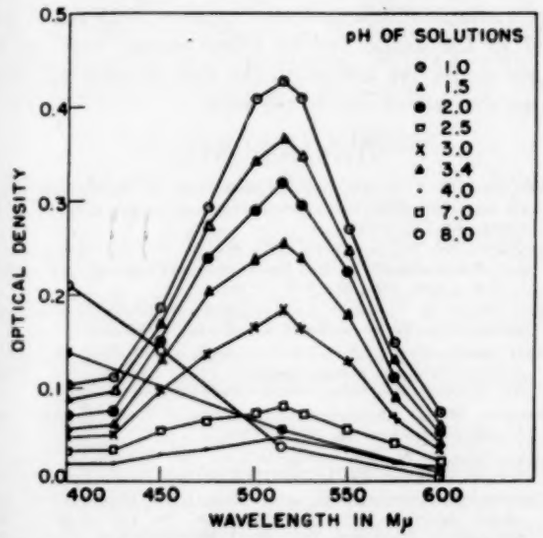

Figure 2. Effect of pH on absorption values of solutions of anthocyanin chloride (monoglycoside).

the same concentration, as the pH is increased, the optical densities are found to decrease up to a pH of 5.0. The absorption maximum occurred at 515 mμ at low pH while with increasing pH the absorption maximum was shifted toward 400 mμ. These variations were found to be less striking for the monoglycoside solutions than with solutions of the diglycoside. The color of the anthocyanidin was found to be faded as the pH increased to 3.0 and at pH's 7.0 and 8.0 for the absorption maxima shifted to 400 mμ in the visible range.

The absorption values of acid solutions of the isolated anthocyanin chloride were recorded by means of the Beckman Quartz spectrophotometer Model DU and plotted in Figure 4. It may be noted that the pigment solution showed an absorption maximum at 280 mμ in the ultraviolet range in addition to that at 515 mμ in the visible range.

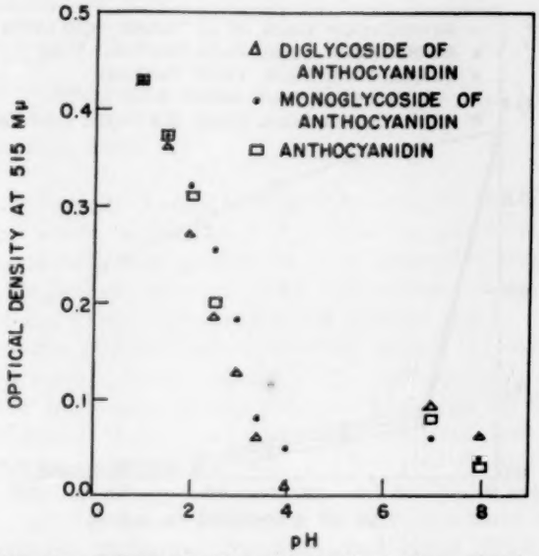

Figure 3. Effect of pH on absorption values of solutions of anthocyanidin, its mono- and diglycosides.

On storage at room temperature, the purified pigment in the solid state formed water-insoluble dark brown residues. The destruction of the pigment in purified pigment solutions upon heating and storage was remarkably high compared to the destruction of the pigment as present in grape juice along with the other juice components including the sugars, pectin, tannins, and organic acids *(15)*.

The results of exposure to ultraviolet radiation indicated that in the initial stages of exposure, the losses of pigment increased with increasing pH values while increased periods of exposure practically destroyed all the anthocyanin pigment. However, these pigment losses were not as appreciable with grape juice samples as those with the anthocyanin chloride solutions. The results presented in Figure 5 indicate that of the 5 substances used, only tannic acid exhibited an extended protective effect on the pigment. In the samples in

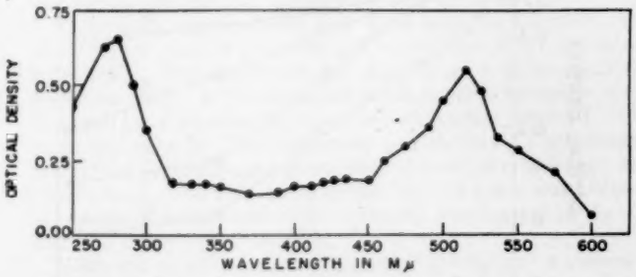

Figure 4. Absorption values of acid solution of anthocyanin chloride over wavelength range of 250-600 mμ.

which tannic acid was added to the pigment solution, it was observed that on exposure to ultraviolet radiation, the optical densities were higher at 400 mμ than those samples treated otherwise. However, exposure of pure tannic acid solution to ultraviolet radiation indicated that the optical density at 515 mμ is negligible and should not cause any interference in the optical density values at the dilutions employed.

The results contained in Figure 6 indicate that the tannin extract from grape juice shows the best pro-

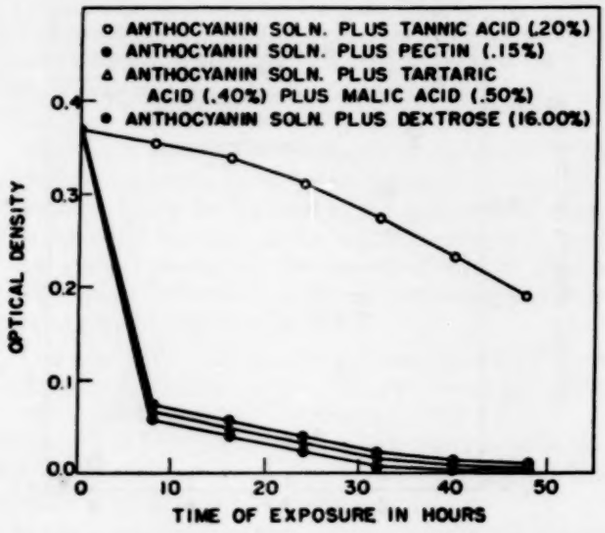

Figure 5. Influence of grape juice components on pigment concentrations of anthocyanin solutions (pH 3.4) exposed to ultraviolet radiation. Pigment concentrations are expressed as optical densities measured at 515 mμ and at pH 1.0 of solutions (1:80).

tective effect for the pigments when the juice samples are exposed to ultraviolet radiation. The results also show that the tannin extract from grape stems is superior to pure tannic acid in protecting the pigments from destruction as a result of exposure of the pigment solutions to ultraviolet radiation. Possibly the juice contains other polyphenolic substances, in addition to tannins, which have effects on the color similar to the tannins. It is equally possible that the anthocyanin pigment and the tannins are present in the grape juice samples in a form not easily destroyed on exposure to ultraviolet radiation.

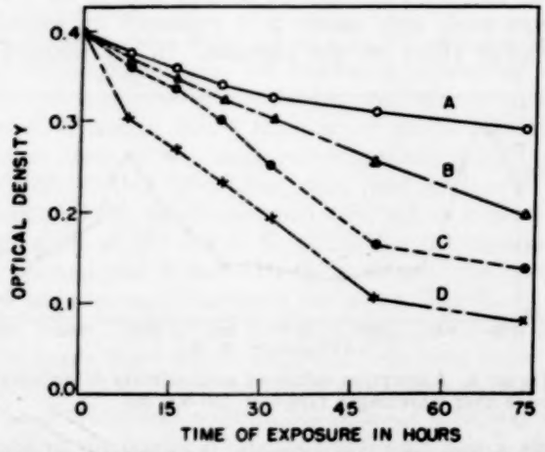

Figure 6. Influence of natural tannin extracts and tannic acid on grape juice free from tannins. Pigment concentrations are expressed as optical densities measured at 515 mμ and at pH 1.0 of solutions (1:80). The four solutions were exposed to ultraviolet radiation. Solution A was grape juice free from tannins and anthocyanin pigments plus extract of tannins and anthocyanin pigments obtained from grape juice; Solution B was grape juice free from tannins and anthocyanin pigments plus pigment solution plus tannin extract from stems (equiv. 0.2% tannin); Solution C was grape juice free from tannins and anthocyanin pigments plus pigment solution plus tannic acid (0.2%); and Solution D was grape juice free from tannins and anthocyanin pigments plus pigment solution.

SUMMARY

Qualitative tests on the methanolic extract of lyophilized grape skins indicated the presence of chlorophyll, water soluble yellow pigments, and carotenes in addition to the anthocyanins. Further support for these observations was drawn from the chromatographic techniques wherein magnesia, sucrose, and alumina were employed as the adsorbents.

The major anthocyanin pigment present in Concord grapes was isolated from fresh ripe skins as the chloride. Attempts to recrystallize the pigment were not successful.

Paper chromatography revealed the presence of 2 water soluble pigments in the methanol HCl (1%) extracts of the lyophilized grape skins. These pigment components formed red and purple bands on drying with R_f values of 0.11 and 0.19 respectively. A hazy red band with an R_f value of 0.56 was also noticed and this band disappeared in the course of approximately 24 hours. These bands with R_f values of 0.56, 0.19, and 0.11 were identified as the anthocyanidin, its mono- and diglycosides, respectively, by hydrolysis and separation procedures.

Exposure of juices and of purified pigments to ultraviolet radiation indicated that tannins, especially those found in the grape and in grape stems, have an important protective action on the anthocyanin which decreases the rate of loss of pigment.

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A Study of Methods for the Measurement of Tomato Juice Color[a,b]

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The use of the Beckman quartz spectrophotometer, the Hunter Color Difference Meter, the Photovolt Reflection Meter, and the Maxwell spinning disc for the measurement of color reflectance in canned tomato juice is described. Results obtained by the different methods are compared and discussed.

Color evaluation in food products has become increasingly important as food technologists place more emphasis on product quality, yet color is one of the hardest quality factors to evaluate. The methods used most widely in the food industry at the present time are based on such color references as plates, spinning discs, and slides. Visual judgment under the best of conditions is arbitrary, and the need for objective methods of color measurement has resulted in the development of reliable photoelectric methods in recent years. In this paper several approaches to color measurement of tomato juice are described and compared. The ultimate authority for comparing the various methods is a measurement of the correlation attained with color scores given by Federal PMA inspectors.

REVIEW OF LITERATURE

The Maxwell spinning disc principle has been used for color measurement for over 100 years. Newhall *(17)*, Nickerson *(18)*, Mitchell *(16)*, Taxner *(22)*, Emmanuele and Mauri *(5)*, and McGillivray *(12)* have described the use of the spinning disc for agricultural products. The last 4 citations refer particularly to tomato products. Carasco *(1)* has reported that the Lovibond tintometer can be used satisfactorily for tomato color work.

McCollum *(15)* attempted to show differences in tomato juices of various grades, using a Beckman quartz spectrophotometer with a reflectance attachment. Although he was unable to demonstrate grade differences, he observed that backing the sample container with white and black papers caused different reflectance values. The reflectance attachment and its use have been described by Pride *(20)* and Gibson *(6)*. Conversion of the reflectance spectrum to the psychophysical values of the International Committee on Illumination (I. C. I.) has been explained in detail by Hardy *(7)*. The relation of I. C. I. values to the Munsell system of color specification has been described by Nickerson *(18)*.

The development of filters that approximate the I.C.I. tristimulus functions *(8)* has made possible the use of photoelectric instruments that give "psychophysical" measurements of color without elaborate calculations. In other words, simple objective physical measurements are now possible that predict the color perceptive response of the human observer. Two instruments making use of these filters were used in the present study. These were the Hunter Color Difference Meter *(9)* and the Photovolt Reflection Meter *(19)*. Kramer *(10)* compared the 2 instruments and concluded that the Color Difference Meter was superior in accuracy and convenience. Younkin *(23, 24, 25)* has published several studies describing the operation and successful application of the Color Difference Meter in tomato color problems. Robinson, Ransford, and Hand *(21)* have described the use of the latter instrument in the measurement and control of color in the canning of tomato juice. Eastmond and co-workers *(3, 4)* made use of the Multipurpose Reflectometer and the G. E. recording spectrophotometer for studying changes in processed and stored foods. Davis *(2)* compared color transmissions of solvent extracts with reflectance readings of tomato puree and concluded that an extraction color index is "not significantly less accurate than existing methods." However, his reflectance work is not described in detail and cannot be calculated or evaluated by the reader.

DIRECTIONAL REFLECTANCE MEASUREMENTS

Beckman quartz spectrophotometer. Directional reflectance can be measured on the Beckman spectrophotometer by means of a special reflectance attachment *(6, 15, 20)*. A Model DU spectrophotometer was used in the present study to measure reflectance in the visible range from 400 to 650 mμ. From the reflectance curves the I.C.I. values for Illuminant C were calculated by the selected ordinate method *(7)*. Thirty ordinates were selected in the majority of cases, although the 10-ordinate method was sufficient to distinguish color differences.

Reflectance measurements were made with the selector switch set at 1.0 for reflectance curves above 10% and at 0.1 for values below 10%. The sensitivity dial was set at 2 turns from the clockwise limit. A freshly scraped magnesium carbonate block was used for a standard and assigned reflectance values of 100% at all wavelengths. The instrument was checked for wavelength calibration by using several emission lines of the hydrogen discharge lamp. The reflectance of the $MgCO_3$ block was checked by means of a standard white Vitrolite block supplied by the National Bureau of Standards.

With the selector switch at "check," the desired wavelength under the hairline, and the phototube switch "off," the dark current knob was rotated to zero the galvanometer needle. The $MgCO_3$ standard was placed in the field of illumination by sliding the drawer of the reflectance attachment to the proper position. With the phototube switch "on," the slit width was adjusted to zero the needle. This procedure set the reflectance of the standard at 100%. The selector switch was set and the sample drawer moved to place the juice in the field of illumination. The percent reflectance at the selected wavelength was indicated by using the "transmission" knob to zero the needle.

In preliminary experiments it was found that higher reflectance values were made when the sample cells were backed with white paper. This confirmed the observation of McCollum *(15)*, and indicated that the sample container should be larger to have reflectance values independent of the type of back-

[a] This work was conducted in conjunction with a cooperative research project between the New York State Agricultural Experiment Station and the Production and Marketing Administration of the United States Department of Agriculture under the authority of the Research and Marketing Act.

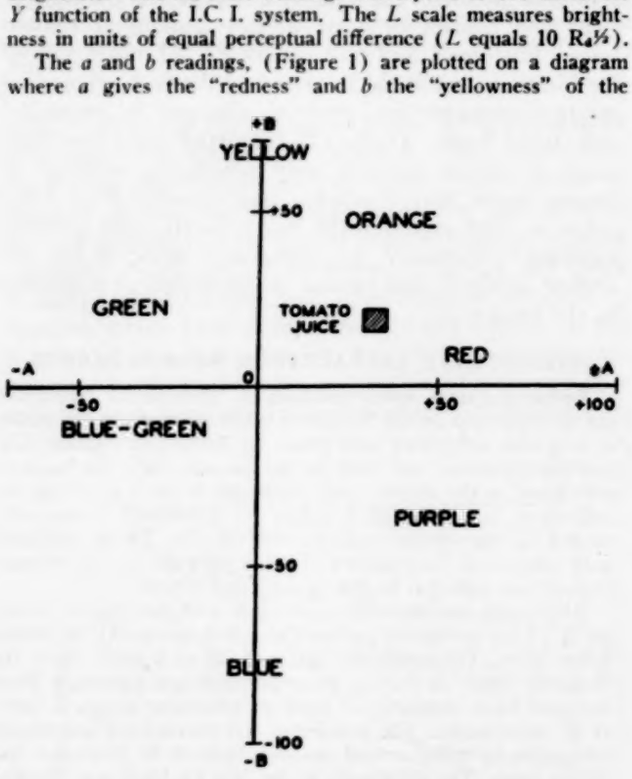
[b] Journal Paper No. 870, New York State Agricultural Experiment Station, Cornell University, Geneva, New York.

ground used. A change in reflectance value with type of cell backing indicates that light is penetrating completely through the cell contents. When light is lost in that manner, the true reflectance value of the sample is not obtained. Further studies on cell dimensions showed that a ¾-inch depth was sufficient for the least opaque juices. Consequently, a Plexiglas sample holder with a cylindrical well measuring 2 inches in diameter and 1 inch in depth was constructed. A modification in the sample drawer was required to accommodate the required cell. The front end of the drawer was extended by making a ¼-inch collar to enlarge the capacity.

In making tomato juice color measurements, the cell was filled to the brim with juice and carefully covered with a slip of clear glass in such a way that no air bubbles were trapped under the glass. This method eliminated variations of the juice level in the sample container as a source of error.

Hunter Color Difference Meter. The Hunter Color Difference meter *(9)* is a photoelectric tristimulus instrument that measures color by the use of three filters that approximate the *X*, *Y*, and *Z* funcions of the I. C. I. system *(8)*. Results are presented in terms of *L* or R_d (brightness), *a* and *b*. The relative values of *a* and *b* determine dominant wavelength, while saturation or "chromaticity" is represented by $(a^2 + b^2)^{1/2}$. The instruments are equipped with R_d and/or *L* scales for the measurement of brightness. The R_d scale readings correspond to 100 times the *Y* function of the I.C. I. system. The *L* scale measures brightness in units of equal perceptual difference (*L* equals 10 $R_d^{1/2}$).

The *a* and *b* readings, (Figure 1) are plotted on a diagram where *a* gives the "redness" and *b* the "yellowness" of the



HUNTER CHROMATICITY DIAGRAM

Figure 1. Hunter color diagram showing the region of tomato juice color.

measured color. The Hunter readings may be converted to I. C. I. coordinates from the formulas:

$$a = 175\ (1.02\ X - Y)/Y^{1/2} \text{ and } b = 70\ (Y - 0.847Z)/Y^{1/2}$$

The I. C. I. data may be readily converted to Munsell hue, value, and chroma by the use of charts. Fortunately, from the standpoint of tomato product color measurement, there is a direct relationship between I. C. I. dominant wavelength and the ratio of *a* to *b* in the tomato color region (Figure 2). This relationship justifies the use of *a*/*b* as an indicator of dominant wavelength, and eliminates the necessity for conversion calculations.

For tomato juice color measurement, the *L* scale was used with small area illumination (about ⅝ × ⅜ inches). The *L*

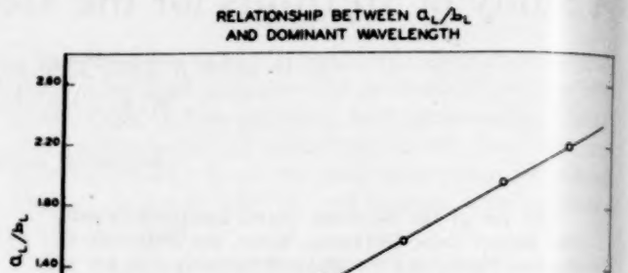


Figure 2. The relation between dominant wavelength and a/b.

scale more closely approximates the response of the human observer than does the R_d scale. With the *L* scale, the reflectance values are expanded over a wider range, and therefore the *L* reading may be set more precisely. This in turn makes the *a* and *b* readings more accurate. All measurements were made with the glass coverplate replaced by a black plastic block having a circular opening 2¼ inches in diameter.

The sample container, of optical-quality Plexiglas, was constructed by using 2-⅝-inch internal diameter Plexiglas tubing to make a cylinder 2½ inches high. A 3½-inch square of flat 1/16-inch Plexiglas was joined to the bottom of the cylinder with a cement made by dissolving shavings of the material in acetone. The cell was filled to within ½ inch of the top for tomato juice color measurement. Color measurements made in this cell agreed with those made in the glass cell described by Younkin *(24)*.

A standard reference tile of red porcelain *(24)* was calibrated on the Beckman spectrophotometer. From the reflectance curve, the I. C. I. values and Hunter readings were calculated, the former by the 30-selected ordinate method *(7)*, and the latter by the formula given above. The Beckman spectrophotometer was used for the standardization because like the Hunter and Photovolt, it has 45°, 0° illumination. Reflectance readings of instruments such as the General Electric recording spectrophotometer cannot be compared since the gloss (or "specular") component of the reflected light is included in the reading. In fact, reflectance readings from differently designed instruments such as the Photovolt and the Hunter cannot be expected to give identical results, since distance from light to sample, sample to photocell, angle of illumination, and arc degrees included in measured light all influence the measurement. The instrument was set to give the correct readings for the standard tile; then a bright red plate (National Bureau of Standards Kitchen and Bathroom tile No. 70) was measured. A log book of daily readings kept on the kitchen and bathroom tile gave a daily check on the reproducibility of the instrument.

A measurement of the precision of the color difference meter was made by determining the color of a sample of tomato juice 20 times at 20-minute intervals during a day. The standard deviation of the *a* to *b* ratio was 0.012, which is equivalent to 0.15 Production and Marketing Administration (PMA) color grade points. The standard deviation of a grader, measured in similar fashion, was 0.94 grade points.

Photovolt Reflectance Meter. The Photovolt Reflectance Meter, Model 610, also measures diffuse reflection. Light strikes the sample surfaces perpendicularly and is viewed at an average angle of 45°. The principle of 0°, 45° reflectance measurement matches the 0°, 45° of the Beckman spectrophotometer's reflectance attachment, as well as the 45°, 0° of the Hunter Color Difference Meter. The Reflection Meter, like the Hunter instrument, makes use of tristimulus filters. However, in the Reflection Meter the separate filter readings are made directly, and from these the *X*, *Y*, and *Z* functions are readily calculated.

Reflection measurements were made in the cells described in the previous section. The standardized red tile employed for the Hunter measurements was also used for the Photovolt meter. In making the reflection readings, the galvanometer needle was set at the highest possible multiple of the calculated reading of the standard for each filter. For example, if the calculated blue filter reading for the standard red plate were 2.0, the galvanometer could be set at 10.0. The true reading for each sample after measurement was then obtained by dividing the observed reading by the proper factor (5 in the above example). In making the blue tristimulus measurement, the readings were very low on the galvanometer scale. A small error in the blue readings, under these conditions, had about 3 times the effect on the resulting color value as did a similar error in the amber or green readings. In fact, it was found that because of the error involved, the use of a formula that excluded blue yielded better correlation with the other instruments than did the theoretically correct formula.

It was necessary, therefore, to increase the sensitivity of the Reflection Meter to differences in the blue component of tomato juice color. This was accomplished in 2 ways. A more sensitive galvonometer was substituted for the one supplied with the instrument. A 25,000 ohm resistance was substituted for the 4,000 ohms of the coarse sensitivity control potentiometer. The latter change had the disadvantage of resulting in less smooth control of sensitivity. For any constant amount of light, the galvanometer deflection rises from zero to approximately 0.9 of the maximum value in the first 120° of rotation of the control. Hence setting of the control is more critical than before for large amounts of light on the photocell. This is not a serious disadvantage since a fine sensitivity control is provided on the instrument. Both changes together increased the sensitivity in the blue range by a multiple of about 3.5.[e]

The relation of the values of the filters used in the Photovolt instrument to the Hunter data is shown by the following conversion formulae *(9)*:

$$a_L \text{ (Hunter)} = 175\ (0.8\ A - G + 0.2B)/G^{1/2}, \text{ and} \quad (1)$$

$$b_L \text{ (Hunter)} = 70\ (G - B)G^{1/2} \text{ where A, G, and B are the amber, green, and blue tristimulus filter readings, respectively.} \quad (2)$$

Since a/b is used as a color index in the Hunter color measurements described here, equation (1) may be divided by equation (2) to give the following:

$$a_L/b_L = 2\ [(A - G)/(G - B)] - 0.5 \quad (3)$$

Therefore, the fraction $(A - G)/(G - B)$, is linearly related to a/b and to dominant wavelength.

The Maxwell spinning disc. The apparatus used in the Maxwell disc determinations consists essentially of a stand supporting the sample holder, the spinning disc, and the comparator eyepiece (Figure 3). The stand rests between 2 supports for a Macbeth Daylight Lamp (Model RCF-30), which is suspended directly above the stand. The table is enclosed on 3 sides by vertical pieces of ⅛-inch masonite painted black. In contrast to the apparatus illuminated by 2 lamps described by Nickerson *(18)* and used by MacGillivray *(12)*, only 1 light source is used to provide identical lighting conditions on the sample spinning disc. Instead of illuminating at a 45° angle, the comparator eyepiece is set at 45°, and the illumination is vertical. This arrangement has been found to be less tiring to the observer.

The Macbeth daylight lamp and Bausch and Lomb comparator eyepiece assure identical color matching conditions at all times. This lamp produces a light with a color temperatûre of 6700° K. and energy distribution corresponding to that of I. C. I. Illuminant C. The light is suspended 8 inches from the surfaces of the sample and spinning disc. The intensity of light reaching those surfaces is 80 foot candles. The comparator eyepiece, mounted at a 45° angle, is so placed that the 2 areas of observation are about ½ inch apart. The observed areas are about 1 inch in diameter. The angle of mounting was found to be critical in determining brightness and chroma, and to some extent, the hue. The 45° angle was chosen to give conditions comparable to those of the photoelectric instrument used in the comparative study.

Sample holders were made from the bottom inch of No. 1 tin cans. The containers measured 1 inch high, 3 5/16 inches in diameter, and contained 125 ml. As a precaution against the possible reflectance of stray light, the inside of each can was painted black. The sample holder support was adjusted so that the surface of any sample or standard could be brought to the level of the spinning disc. The 4 Munsell discs and the calibrated backing were mounted on a small fan motor and base, with the discs in a horizontal position. The speed of the motor was sufficient to avoid color flickering. The motor base was held in place by adjacent screws covered with rubber tubing to prevent vibration.

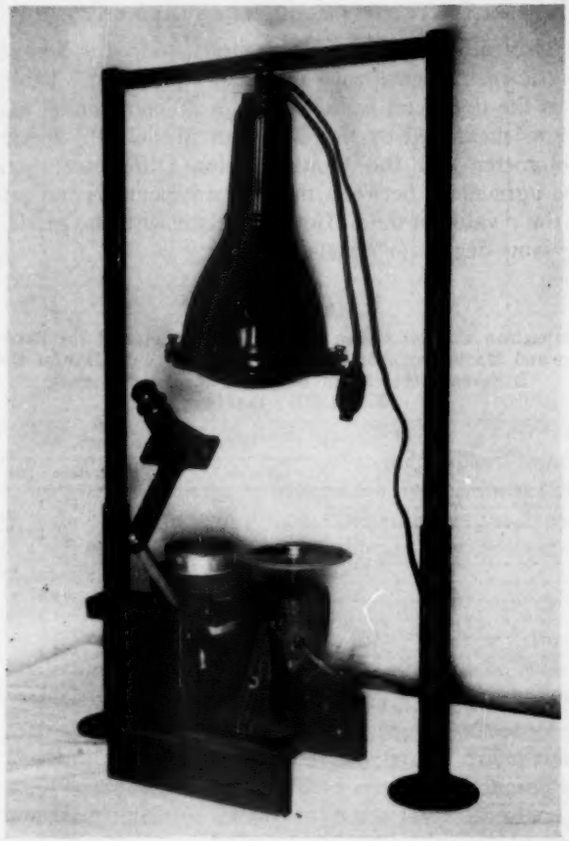


Figure 3. The Maxwell spinning disc apparatus. The black background is removed to show detail.

For measuring commercial tomato juice color, Munsell discs with Atlas notations of 5R 2.6/13 (Red), 2.5 YR 5/12 (Yellow), N6 (Gray), and N1 (Black) were used. The production numbers were 3036, 2476, 1174, and 2373, respectively. When measuring standard tomato papers and dark juices, a darker gray (N4) was substituted for the N6. The room was darkened so that the only available light was the daylight lamp. The 3 color dimensions, hue, value, and chroma, are matched in that order. The hue is first approximated by adjusting the proportion of red to yellow: the value (brightness) was then adjusted by increasing or decreasing the proportion of gray to black; and then the chroma was adjusted by changing the proportion of red and yellow to gray and black. The process was then repeated until a satisfactory match was obtained. The disc settings were obtained by recording the combinations that were too red and too yellow; too light and too dark; too weak and too strong. Thus the range may be rapidly narrowed. However, the work is tedious and requires a high degree of concentration.

Hue may be calculated most accurately by the I. C. I. method *(18)*. The 4 discs are measured on a Beckman spectrophotometer

[e] Photovolt Lumetron Model 402-E, with reflectance attachment, achieves this sensitivity, and may be adapted for tomato juice color measurement.

and their I. C. I. values computed. The X, Y, and Z fractions of each disc are then multiplied by the percentage of the disc used in obtaining the match. The sums of the separate X, Y, and Z values thus obtained define the color.

A simple method of expression, though theoretically less accurate, is the ratio of red to yellow (R/Y) in the matching mixture. This method is less accurate because it ignores the effect of chroma and brightness on the hue. For practical purposes, however, it has proven to be suffiicently accurate and far more convenient for measuring tomato juice color.

RESULTS OF COLOR MEASUREMENTS

Color measurement was accomplished by the 3 photoelectric instruments with excellent agreement. Table 1 gives the dominant wavelengths of 28 commercial samples as measured by the Beckman Model DU spectrophotometer and the Hunter Color Difference meter. The agreement between the 2 instruments is indicated by the r value of 0.93. Both correlate with the grade to the same degree (r equals 0.78).

TABLE 1

Comparison of color scores assigned by a grader of the Production and Marketing Administration (PMA) with Hunter Color Difference Meter and Beckman spectrophotometer (Model DU) readings [4]

Color Score (y) (1949)	Dominant wave length, mμ	
	Hunter (a)	Beckman (b)
28	600.2	595.3
26	598.6	593.6
23	599.4	594.8
26	599.1	594.7
27	601.1	595.8
27	599.2	595.0
27	598.8	594.2
27	599.5	595.6
27	600.9	595.4
24	597.3	593.3
27	600.2	594.8
27	600.7	595.3
22	595.2	591.8
30	602.5	596.8
28	599.8	595.5
27	598.6	594.7
27	598.5	594.9
28	601.7	597.0
26	601.3	596.5
24	597.2	593.8
26	599.8	595.0
28	600.8	596.5
26	599.8	595.5
25	597.9	593.8
27	600.2	595.7
27	600.4	595.9
25	598.4	593.8
27	600.2	595.2

[4] Values for r for ab, ya, and yb are 0.93, 0.78, and 0.78, respectively.

Figure 4 shows typical reflectance spectra of grade A and grade C tomato juices measured on the Beckman spectrophotometer. The curves are often difficult to interpret by visual inspection; however, good tomato juice color is relatively brighter in the region above 600 mμ, and low in the region around 550 mμ. The small peak in the curve in the yellowish-green region is in all probability caused by the presence of a chlorophyll derivative. Juice made from under-ripe tomatoes is characterized by a relatively high reflectance at 550 mμ.

The Photovolt Reflection Meter, Model 610, as modified for these studies, was accurate in measuring color. For this comparison, a series of 20 commercial and pilot plant samples of tomato juice manufactured in 1950 were used (Table 2). The samples were chosen to give a wide range of color quality. Correlation with the in-

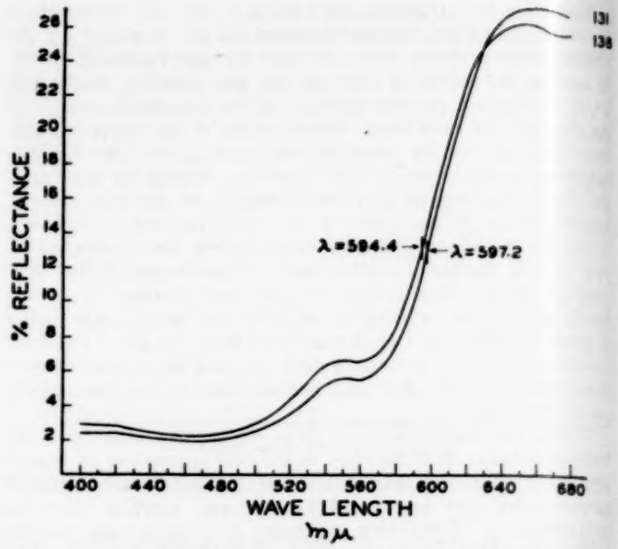


Figure 4. Reflectance curves of Grade A and Grade C tomato juice as measured by Beckman quartz spectrophotometer (Model DU).

spector's color score was equally good for the Photovolt (r equals 0.92) and for the Color Difference Meter (r equals 0.90).

Color Difference Meter data was obtained on over 800 samples from the Experiment Station pilot plant and from commercial canneries. The Munsell hue was calculated in 2 ways: first using the a and b readings only at value 3.0, and secondly by including the proper correction for value. Table 3 gives the correlation of these 2 methods of presenting hue as well as of the a to b ratio with the PMA grader's scores. To clarify the distinction between these 3 methods of presenting color: a to b ratio corresponds to dominant wave length; Munsell hue at value 3.0 compensates for the psychological effect of differences in chroma on the appearance of that dominant wavelength; and the Munsell hue corrected for value compensates for the effect of brightness as well as chroma on the appearance of a dominant wavelength.

For the final testing of the Maxwell disc method of color measurement, the same 20 samples were used that were mentioned previously in the comparison of the Photovolt and Hunter instruments. The 3 different operators made entirely independent color measurements on these samples. From the composition of the matching discs the Red to Yellow ratio (R/Y index) was calculated, and by the use of the I. C. I. method of calculation *(18)* the hues at value 3.0, and the hues adjusted for value were calculated. The following comparison of the correlations obtained with PMA color grade (r values) shows that none of the 3 methods used for expressing the results is consistently superior:

	Operator 1	Operator 2
R/Y index	+0.908±0.021	+0.923±0.024
Hue (at value 3.0)	−0.926±0.039	−0.916±0.020
Hue (corrected for value)	−0.860±0.027	−0.932±0.022

Although these differences are not large enough to be highly significant, they seem to indicate that the effect of brightness on hue, a psychological effect, is different with different observers. Operator No. 1 gives better

TABLE 2
Comparison of methods for color measurement

Sample	PMA Color Grade (1951)	Color Difference Meter					Photovolt				Munsell Disc R/Y		
											Operator		
		L	a_L	b_L	a/b	Hue*	A	G	B	A—G / G—B	1	2	3
1	24	28.9	20.2	14.2	1.42	12.31	11.34	7.04	2.54	0.96	2.91	3.10	3.00
2	26	28.0	18.3	12.2	1.50	11.80	10.34	6.47	2.92	1.09	3.68	3.97	3.70
3	23	27.8	17.8	13.4	1.33	12.88	10.41	6.54	2.39	0.93	2.64	2.66	2.31
4	28	24.6	19.0	11.1	1.72	10.52	8.83	5.32	2.23	1.14	5.62	6.60	4.19
5	24	27.8	18.6	13.2	1.40	12.41	10.21	6.38	2.27	0.93	3.05	3.30	3.00
6	25	26.4	19.0	12.6	1.51	11.83	9.47	5.78	2.13	1.01	4.18	4.30	3.35
7	27	27.1	17.4	11.8	1.47	11.82	9.55	6.08	2.78	1.05	3.50	4.22	3.60
8	25	28.6	20.6	14.0	1.47	12.30	11.62	7.05	2.39	0.98	2.74	3.20	2.68
9	28	27.6	22.1	12.5	1.77	10.70	11.23	6.68	2.79	1.17	4.47	5.25	4.76
10	21	29.0	17.2	14.0	1.23	13.50	10.08	6.60	2.61	0.87	2.00	2.26	2.37
11	21	29.6	19.2	14.2	1.35	12.91	11.52	7.30	2.84	0.95	1.86	2.34	2.50
12	27	27.8	22.8	12.6	1.79	10.63	11.65	6.86	2.73	1.16	4.53	5.19	4.71
13	23	29.8	19.2	14.3	1.34	12.96	11.98	7.57	2.98	0.96	2.00	2.22	2.57
14	23	29.0	17.2	13.9	1.24	13.45	10.34	6.70	2.58	0.88	2.00	2.40	2.54
15	26	29.0	22.8	13.6	1.66	11.39	12.23	7.30	2.71	1.07	3.65	3.88	4.00
16	28	28.9	18.9	11.6	1.63	11.05	9.42	5.80	2.55	1.11	4.00	5.64	4.41
17	27	27.4	18.5	12.0	1.54	11.55	9.68	5.99	2.61	1.09	3.56	4.34	3.94
18	30	26.2	20.4	11.4	1.78	10.29	9.83	5.87	2.49	1.17	5.69	6.64	6.08
19	25	27.3	18.5	13.0	1.42	12.33	9.98	6.19	2.31	0.98	3.15	3.37	3.08
20	27	29.1	22.4	13.3	1.69	11.32	12.29	7.23	2.71	1.12	4.10	3.98	4.26
Correlation coefficient†					0.90	—0.94				0.92	0.91	0.90	0.92

* Hue at value of 3.0 not corected for differences in value.
† Correlation with PMA color grade assigned by inspector.

correlation before the correction for brightness is applied to the hue. Operator No. 2, on the other hand, gives better correlation after the adjustment for brightness and chroma have been applied. Since the R/Y index is intermediate in both cases, is easily calculated, and has been found to give good results by several investigators from this laboratory, it is used in Table 2 to compare with the photoelectric methods of color measurement. The average R/Y index of the twenty samples for Operator No. 1 was 3.47, for Operator No. 2, was 3.94, and for No. 3, was 3.55. Although Operator No. 2 used a higher proportion of red in his disc to match the tomato juice, his independent correlation with the grades was just as good. Equations for color grades were as follows for the 3 operators:

$$\text{Color score} = 19.3 + 1.74\ (R/Y)\ \text{(Operator No. 1)}.$$
$$\text{Color score} = 19.9 + 1.39\ (R/Y)\ \text{(Operator No. 2)}.$$
$$\text{Color score} = 18.3 + 2.00\ (R/Y)\ \text{(Operator No. 3)}.$$

Examination of the above equations shows that each operator must have his own prediction equation for satisfactory work. Substitution of Operator No. 2's results in No. 1's equation may give scores a whole grade point too high. The calculated hues show the same type of discrepancy. Operator No. 2 used a redder hue to match the color of tomato juice than did No. 1 in each of the 20 samples. The use of the spinning disc presents disadvantages other than individual operator differences. The discs are of necessity exposed to the air, and rapidly become worn and dirty. Furthermore, replacement discs may not be of the same production number, and each new disc must therefore be recalibrated by a spectrophotometer.

TABLE 3
Correlation of Hunter Color Difference Meter measurements with PMA color grade of tomato juice

Year	Type of sample	Number of samples	Correlation of color grade assigned by PMA inspector with		
			a/b	Calculated hue at value of 3.0[a]	Calculated Munsell hue[b]
1949	Commercial	28	+0.771	—0.799	—0.831
1949	Pilot plant	85	+0.905	—0.853	—0.943
1950	Commercial	30	+0.838	—0.929	—0.919
1950	Pilot plant	684	+0.964	—0.958	—0.980

[a] Hue corrected for chroma, not value.
[b] Hue corrected for chroma and value.

DISCUSSION

The color of tomato juice must be caused by its chemical composition and its physical condition. Carotenoid pigments, especially carotene and lycopene, chlorophyll derivatives, and other pigments may have a direct bearing on the color. However, the dispersion of these pigments, the volume of the solids in the juice, presence of other pigments, and types of crystallization could conceivably influence the color produced by the pigments. For example, lycopene, the red pigment in tomato juice, must be carried on the suspended solids of the juice since it is insoluble in water. Two juices of the same lycopene content, but of widely differing insoluble solids contents would present quite different appearance. These considerations help to explain the poorer correlation obtained between chemical methods and visual ratings, as compared to reflectance measurements related to visual ratings.

Since the color caused by an insoluble pigment such as lycopene depends on other factors than the quantity present, a method other than quantitative determination of pigment concentration is to be preferred. The best possible available approach is analysis of the reflected light. Spectrophotometric analysis has been available for many years, but calculations to show the relationship of spectrophotometric data to the response of the human eye are tedious unless one has available an instrument such as the G. E. recording spectrophotometer and the integrating librascope for computing I. C. I. color values. The development of tristimulus filters *(8)* has made possible the development of instruments that are rapid, accurate, less expensive, and more compact.

TABLE 4

Comparison of Maxwell color disc measurements on twenty tomato juice samples by two operators

Sample number	Operator No. 1						Operator No. 2					
	R[1]	Y	G	B	R/Y	Hue	R	Y	G	B	R/Y	Hue
1	67	23	0	10	2.91	11.1	74.5	24	0	1.5	3.10	11.0
2	70	19	0	11	3.68	10.9	75.5	19	1	4.5	3.97	10.6
3	58	22	0	20	2.64	11.4	62.5	23.5	0	14	2.66	11.2
4	73	13	0	14	5.62	10.7	82.5	12.5	0	5	6.60	10.4
5	64	21	0	15	3.05	11.1	72.5	22	0	5.5	3.30	10.9
6	71	17	0	12	4.18	10.9	77.5	18	0	4.5	4.30	10.7
7	63	18	1	18	3.50	11.0	76	18	1.5	4.5	4.22	10.4
8	63	23	0	14	2.74	11.3	72	22.5	0	5.5	3.20	11.0
9	76	17	0	7	4.47	10.8	84	16	0	0	5.25	10.4
10	56	28	0	16	2.00	11.4	64.5	28.5	0	7	2.26	11.2
11	54	29	0	17	1.86	11.6	65.5	28	0	6.5	2.34	11.1
12	77	17	0	6	4.53	10.7	83	16	0.5	0.5	5.19	10.4
13	58	29	0	13	2.00	11.3	64.5	29	0	6.5	2.22	11.2
14	56	28	0	16	2.00	11.4	66	27.5	0	6.5	2.40	11.0
15	73	20	0	7	3.65	10.9	79.5	20.5	0	0	3.88	10.7
16	64	16	0	20	4.00	11.0	79	14	0.5	6.5	5.64	10.4
17	64	18	0	18	3.56	11.1	76	17.5	0.5	6	4.34	10.6
18	74	13	0	13	5.69	10.7	83	12.5	0.5	4	6.64	10.3
19	63	20	0	17	3.15	11.1	72.5	21.5	0	6	3.37	11.0
20	78	19	1	2	4.10	10.7	79.5	20	0.5	0	3.98	10.6

[1] Specifications:

	Atlas Notation	Production No.
R = Munsell disc area	5R2.6/13 (red)	3036
Y = Munsell disc area	2.5 YR 5/12 (yellow)	2476
G = Munsell disc area	N6 (gray)	1174
B = Munsell disc area	N1 (black)	2373

The existence of many systems for presenting color dimensions is confusing to the novice in the field. For the purpose of this discussion, the I. C. I. system (dominant wavelength, brightness, and purity) and the Munsell system (hue, value, and chroma) must be reconciled. Hue and dominant wavelength, although terms for the same color dimension, are not identical. Munsell hue changes not only with dominant wavelength, but is affected slightly by chroma and value. This is caused by a psychological effect on the observer. Colors of the same dominant wavelength are interpreted as slightly different hues when chroma and value differ. In the tomato color region, according to the Munsell system, higher chroma is interpreted as yellower hue, while higher value is interpreted as a redder hue. From these considerations, we may conclude that the Munsell system is probably the best form of color data expression, since it more nearly corresponds to the interpretation of the majority of human observers However, as is demonstrated by the experiments reported here with three different operators of the Maxwell spinning disc, individual observers may vary in their interpretation. When samples are similar in value and chroma the above considerations are eliminated and the dominant wavelength (or Hunter *a* to *b* ratio) is satisfactory and much simpler to use. Commercial tomato juice samples of widely different origin and manufacturing method may vary greatly in value and chroma, and calculation of hue may be preferable in such cases.

The *a* to *b* ratio of the Color Difference Meter was related to the fraction (A — G)/ (G — B) for the Reflection Meter according to formula *(3)* given above. Thus it is seen that the two are related linearly. Since a_L/b_L is related to dominant wavelength, the function (A — B)/(G — B) too, must be related to dominant wavelength. The choice of photoelectric instruments will be influenced by the intended use, and the relative importance of rapidity and ease of operation. The Color Difference Meter was the more rapid and convenient instrument tested, although with slight modifications the Photovolt instrument, Model 610 proved to be capable of as great accuracy. The Maxwell spinning disc is less objective than the photoelectric instrument, and its use is more tedious and time consuming.

SUMMARY

The use of the Maxwell spinning disc, the Beckman quartz spectrophotometer (Model DU), the Photovolt Reflection Meter, and the Hunter Color Difference Meter for the measurement of reflected color of tomato juice is described. The various methods are compared and evaluated on the basis of correlation with grades given by Production and Marketing Administration inspectors. Of the methods tested, the Color Difference Meter was found to be the most rapid, although the Photovolt instrument was equally accurate under the conditions described here.

In the use of the Color Difference Meter for grading purposes, the ratio of the *a* to *b* readings is a convenient and accurate method of expressing the color of tomato juice within the brightness and chromaticity ranges normally encountered.

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Detection of Commercial Dodecylbenzene Sodium Sulfonate in Canned Foods

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The increasing use of surface active agents in the cleaning and preparation of foods makes desirable, methods of detecting their presence in the products offered for human consumption. Two methods are presented for the extraction of commercial dodecylbenzene sodium sulfonate from food products. The extracted surfactant may be detected and roughly estimated by either of two proposed procedures. Toxic amounts are many times greater than the 25 p.p.m. that can be detected.

The properties of the surface active agents in common use indicate their probable utility for many purposes in the food industry *(2)*. The removal of dirt from the surface of leaf vegetables, the cleaning and "flotation" of vined canning peas, the lye peeling of fruits, and the use of these agents as emulsifiers and antistaling agents have been the subjects of several papers *(4, 5, 7)*. The use of surface active agents to promote sanitation in food processing plants is another potential utilization for these materials *(6)*.

Chemical agents used to assist in removal of extraneous matter from foodstuffs or to assist in processing frequently may either be adsorbed in the operation or carried through mechanically. Absence of such agents from foodstuffs as consumed eliminates potential complaint. Consequently, satisfactory methods for their extraction and estimation are necessary steps in extending the application of these useful materials.

The present paper is concerned only with commercial dodecylbenzene sodium sulfonate (Santomerse No. 3) and with its detection in food products which have been processed using it either for cleaning purposes or as an aid in lye peeling. However, the methods presented are probably applicable to similar anionic surfactants.

A method which can be used successfully in the analysis of these agents must provide for separation of the surfactant from the food, have high sensitivity, accuracy, and reproducibility. The procedure of Jones *(3)* was modified slightly for use in determining the Santomerse No. 3 in canned peas, and in canned peaches and apricots which had been lye-peeled with the aid of this surfactant. The procedure described by Barr, Oliver, and Stubbings *(1)* using bromophenol blue indicator was used in the determination of the Santomerse No. 3 in washed asparagus. The application of these analytical methods required the development of extraction procedures, following which they could then be applied,

EXPERIMENTAL

Extraction Procedure No. 1

Separate the juice and pulp by pouring the contents of the can onto a No. 8 or No. 10 sieve or similar screen and allow to drain. Filter the juice through a layer of cotton placed in a funnel. If the total surface active agent is desired weigh the pulp and obtain the density of the liquid portion by weighing a measured volume.

Preparation of the juice. Pipet 20 ml. of the juice into a 250 ml. beaker. Add 80 ml. of absolute alcohol, cover with a watch glass, bring to a boil and simmer about 45 minutes. Cool and filter through a sintered glass funnel of medium porosity into a 250-ml. filter flask. Transfer the solids to the filter and rinse the beaker and solids with 80% (vol./vol.) alcohol until the rinsings are nearly colorless.

Transfer the filtrate to a 400-ml. beaker, cover, and evaporate to 50 ml. Rinse the cover and sides of the beaker with hot water and dilute to 100 ml. Add 0.5 ml. of hydrochloric acid and evaporate to about 35 ml. Cool and filter into a volumetric flask. Rinse with several small portions of water and dilute to 50 ml.

Preparation of the pulp. Cut the pulp from above into small pieces and mash thoroughly in a mortar and pestle. Weigh 20 g. ± 0.1 g. of the well mixed pulp into a 600-ml. beaker and add 300 ml. of 80% alcohol. Cover, bring to a boil, and simmer 45 minutes, stirring occasionally. Cool and filter through a sintered glass funnel of medium porosity into a 500-ml. filter flask. Transfer the solids to the filter and rinse thoroughly with 80% alcohol.

Transfer the filtrate to an 800-ml. beaker, cover, and evaporate at a gentle boil to 75 ml. Rinse the cover and sides of the beaker with hot water and dilute to 150 ml. Add 1 ml. of hydrochloric acid and evaporate to 75 ml. Cool, filter into a volumetric flask and dilute to 100 ml. with water.

Procedure No. 2

Separate the juice and pulp as described in Procedure No. 1.

Preparation of the juice of vegetables. Pipet 20 ml. of juice into a large test tube (2.5 × 19 cm.). Add 1 ml. of about 7*N*. sodium hydroxide. Place the sample in a boiling water bath

for 1.5 hours to precipitate materials that interfere with the development of the methylene blue color complex. Prevent the volume from falling below 15 ml. Remove from the water bath, cool, and filter into a small separatory funnel. Neutralize with hydrochloric acid. The sample is ready for the Santomerse determination by the modified Jones method.

Preparation of the juice of fruits. No preparation other than dilution with water was necessary. Add 14 ml. of water to 10 ml. of the juice in a separatory funnel and proceed with the modified Jones method.

Preparation of the pulp. Cut the pulp of fruit or vegetables into small pieces and place in a soxhlet extraction apparatus without a thimble but with the outlet loosely plugged with cotton. Extract with 100 ml. of 95% alcohol containing 1 ml. of HCl until the color is nearly gone from the pulp (1.5 to 2 hours).

Evaporate the alcoholic extract to about 30 ml., add 30 ml. of water, neutralize with sodium hydroxide and filter. Rinse several times and dilute to 100 ml. Remove the chlorophyll by four 20-ml. extractions in a separatory funnel with low boiling petroleum ether (Skellysolve F). Wash the petroleum ether with water and add the washings to the main sample solution. Evaporate the solution to about 75 ml., cool, and filter into a volumetric flask. Rinse and dilute to 100 ml.

Determination of the Surface Active Agents

Method No. 1. This is the method of Barr, Oliver, and Stubbings *(1)* using bromophenol blue indicator.

The sample containing up to 15 mg. of surface-active agent, prepared by one of the above extraction procedures, is divided into equal portions and each transferred to an 8-ounce glass-stoppered bottle. Adjust the solution to a pH of 6 (4 to 8 is permissible) using a pH paper. Dilute to 120 ml., add 50 ml. of C. P. chloroform and 10 drops of bromophenol blue solution (0.04% in 20% alcohol). Titrate the samples with approximately 0.001 *M*. cetyltrimethyl ammonium bromide (CTAB) previously standardized against Santomerse No. 3. The CTAB reacts preferentially with anionic surfactants, after which it forms a complex with the bromophenol blue which is soluble in chloroform, giving the chloroform layer a greenish-blue color at the end point. Excess CTAB intensifies the blue color in the chloroform layer.

Method No. 2. The method of Jones *(3)* for determining organic sulfate and sulfonate surfactants was modified slightly by: 1, making 3 extractions; 2, washing with acidified water; 3, using a blank for reference solution; and 4, by preparing a standard curve with Santomerse No. 3.

An aliquot of the prepared sample solution containing up to 0.4 mg. of Santomerse No. 3 is transferred to a small separatory funnel, diluted to 24 ml., acidified with 4 to 6 drops of hydrochloric acid, and reacted with 1 ml. of 0.1% aqueous methylene blue chloride (1.0 mg.) by gently shaking for 1 minute. The Santomerse-methylene blue complex is extracted 3 times with 25-ml. portions of chloroform. Each extract is drawn into a second separatory funnel and washed with acidified water (1 ml. of hydrochloric acid per liter). The chloroform layers are run into a volumetric flask through a cotton plug in a funnel. The combined chloroform extracts are diluted to 100 ml. with chloroform.

The color intensity is measured in a spectrophotometer with an incident light beam of 652 mμ wave-length. (We used a Coleman, Model 14, spectrophotometer). A blank substituting distilled water for the sample is run concurrently with the sample and used as the reference solution. A standard curve is prepared by reacting known amounts of Santomerse No. 3, and sample values are read from the curve.

DISCUSSION

Development of procedures. In this work the juice and pulp from the canned products were separated and analyzed separately. However, if extraction procedure No. 1 is used, the entire can of food can be mixed in a blendor and samples of the levigated material taken for analysis.

As is common with foodstuffs, many difficulties were encountered in the extraction of the surfactants. Materials naturally present in foods and which give a positive test are extracted with the surfactant. This makes surfactant-free control samples necessary. On the other hand, there seem to be substances present in foods that combine with the surfactant or prevent its complete extraction, thereby giving low results.

With peas a green color was formed with the methylene blue. The interfering material was precipitated from the juice by making it alkaline and heating it in a water bath. Extraction with Skellysolve F removed most of this material from the pulp samples. Peach pulp yielded a dark brown extract which also formed a green color with methylene blue. Evaporation of the acidified extract on the steam bath precipitated nearly all of the interfering material.

Addition of Santomerse No. 3 to the food products. Samples of the food products which contained no added surfactants were secured to serve as control samples. Accurately measured amounts of standard Santomerse No. 3 solutions were added to juice samples and mixed thoroughly before the extractions were made. Known amounts of the standard Santomerse No. 3 solutions were added to the weighed pulp samples and let stand overnight to permit thorough infiltration before the extractions were made.

Comparison of the Jones method with that of Barr, Oliver, and Stubbings for determining Santomerse No. 3. The modified Jones *(3)* method has the advantage of being somewhat more sensitive than that of Barr, Oliver, and Stubbings *(1)*. As little as 5 p.p.m. can be detected by the Jones method, whereas 25 p.p.m. was the lowest concentration tried by the other method. The aliquot chosen for the development of the methylene blue color complex must lie between 0.05 mg. and 0.4 mg. of active surfactant. The Jones method also suffers from greater interference in the formation of the color complex by substances extracted from the food along with the Santomerse No. 3, and from adsorption of the color complex on the walls of the volumetric flask in which it is diluted to a standard volume. The cationic titration method is easier to manipulate, requires less time, and covers a wider range (0.2 mg. to 8 mg. of active Santomerse No. 3).

The combination of extraction procedure No. 1 and determination of the Santomerse No. 3 by cationic titration is our choice of the several procedures presented.

RESULTS

Table 1 shows results obtained with asparagus. These samples were prepared by extraction procedure No. 1 and the Santomerse determined by method No. 1. It is apparent that when Santomerse is added to a sample of canned asparagus the recovery, at best, is some 50 to 60% of the amount added. However, the values obtained with one pulp sample (P-6) show that when 0.5 mg. is added to a 20 g. sample it is easily detected. This amount is equivalent to 0.0025% or 25 p.p.m. and is far below a toxic amount. Santomerse is of a low order of toxicity, being in the range of 400 mg. per kg. Samples of commercially canned asparagus which had

TABLE 1
Determination of Santomerse No. 3 in asparagus

Sample	Santomerse No. 3 added to sample	Aliquot titrated	Volume of CTAB[a]	Net Vol. CTAB	Santomerse No. 3[c] in aliquot		Recovery
					Added	Found	
	ml.	*ml.*	*ml.*	*ml.*	*mg.*	*mg.*	%
Juice[b]							
J-1	0.0	10.0	0.11, 0.12	.....	.....	.....	.....
J-2	0.0	25.0	0.22, 0.225	.....	.....	.....	.....
J-3	0.0	25.0	0.16, 0.17	.....	.....	.....	.....
J-4	1.0	10.0	0.35, 0.34	0.23, 0.22	0.20	0.10	50
J-5	2.0	25.0	1.23, 1.38	1.04, 1.19	1.00	0.50	50
Pulp							
P-1	0.0	25.0	0.12, 0.18	.....	.....	.....	.....
P-2	0.0	50.0	0.27, 0.28	.....	.....	.....	.....
P-3	0.0	50.0	0.25, 0.29	.....	.....	.....	.....
P-4	1.0	25.0	0.48, 0.52	0.33, 0.37	0.25	0.16	63
P-4	1.0	50.0	0.84	0.57	0.50	0.26	52
P-5	1.0	50.0	0.84, 0.87	0.57, 0.60	0.50	0.27	54
P-6	0.5	50.0	0.42, 0.46	0.15, 0.19	0.25	0.08	32
P-7	2.0	50.0	1.26, 1.25	0.99, 0.98	1.0	0.45	45

[a] 1 ml. of the CTAB solution is equivalent to 0.45 mg. of Santomerse No. 3.
[b] Juice and pulp prepared by extraction procedure No. 1.
[c] Determination of Santomerse by method No. 1.

been washed with Santomerse showed no detectable amounts of Santomerse retained in them.

Tables 2 and 3 give the results obtained with peaches and apricots. Table 3 shows that 0.05 mg. of Santomerse No. 3 added to 10 ml. of juice (5 p.p.m.) and 1.0 mg. added to 20 g. of pulp (50 p.p.m.) are readily detected, with 75 to 85% recovery in the case of the juice and 50% recovery with the pulp. These values are not the lowest limit for detection but were the lowest tried. Samples of commercially canned peaches and apricots which had been lye-peeled with the aid of Santomerse were tried but not listed in the tables. By these methods they showed 0 to 0.8 p.p.m. retained in the juice, and 0 to 1.7 p.p.m. in the pulp.

Table 4 represents the results of canned peas. No Santomerse was used in preparing these peas for canning. No Santomerse was added to the control can but 100 mg. of Santomerse No. 3 was added to the sample can. There were 86.0 g. of pulp and 50.8 ml. (52.7 g.) of juice in the sample can. After separation 17.0 g. of pulp were weighed out for each pulp sample. This was the first canned food tried and the techniques were not as well worked out as later, which may partially account for the low recovery of 26%. However, the detection of this low concentration of 0.007% (70 p.p.m.) was definite. It is interesting to note that the Santomerse was not evenly distributed between juice and pulp but was concentrated in the pulp.

TABLE 2
Determination of Santomerse No. 3 in peaches

Sample	Santomerse No. 3 added to sample	Aliquot for determination	Santomerse No. 3[f] in aliquot		Recovery
			Added	Found	
	mg.	*ml.*	*mg.*	*mg.*	%
Juice[d]					
J-10	0.0	10.0	0.0	0.022	....
J-11	0.0	10.0	0.0	0.013	....
J-12	0.0	10.0	0.0	0.020	....
J-13	0.0	10.0	0.0	0.007	....
J-14	0.10	10.0	0.10	0.081	81
J-15	0.20	10.0	0.20	0.150	75
J-16	0.20	10.0	0.20	0.153	76.5
J-17	0.20	10.0	0.20	0.150	75
Pulp[e]					
P-10	0.0	20.0	0.0	0.015	....
P-11	0.0	20.0	0.0	0.013	....
P-12	0.0	20.0	0.0	0.015	....
P-13	0.75	20.0	0.15	0.107	71
P-14	1.0	20.0	0.20	0.109	54
P-14	1.0	20.0	0.20	0.100	50

[d] Juice prepared by extraction procedure No. 2.
[e] Pulp prepared by extraction procedure No. 1.
[f] Determination of the Santomerse by method No. 2.

TABLE 3
Determination of Santomerse No. 3 in apricots

Sample	Santomerse No. 3 added to sample	Aliquot for determination	Santomerse No. 3[i] in aliquot		Recovery
			Added	Found	
	mg.	*ml.*	*mg.*	*mg.*	%
Juice[g]					
J-20	0.0	10.0	0.00	0.003	....
J-21	0.0	10.0	0.000	0.005	....
J-22	0.0	10.0	0.000	0.000	....
J-23	0.05	10.0	0.05	0.043	86
J-24	0.05	10.0	0.05	0.042	84
Pulp[h]					
P-20	0.0	20.0	0.00	0.014	....
P-20	0.0	20.0	0.00	0.014	....
P-21	1.0	20.0	0.20	0.099	49.5
P-21	1.0	20.0	0.20	0.104	52.0

[g] Juice prepared by extraction procedure No. 2B.
[h] Pulp prepared by extraction procedure No. 1B.
[i] Determination of the Santomerse by method No. 2.

TABLE 4
Determination of Santomerse No. 3 in peas

Sample	Aliquot for determination	Santomerse No. 3 equivalent in aliquot		Santomerse No. 3[m] in all of juice or pulp
		Total	Net	
	ml.	*mg.*	*mg.*	
Juice[k]				
Control 1	15	0.026	.....	.....
Control 2	20	0.033	.....	.....
Sample 3	20	0.193	0.16	0.40
Sample 4	20	0.190	0.16	0.40
Pulp[l]				
Control 5	2	0.023	.....	.....
Control 6	3	0.020	.....	.....
Sample 7	2	0.125	0.102	25.8
Sample 8	3	0.174	0.154	26.0

[j] Total Santomerse No. 3 added to can is 100 mg. Total Santomerse No. 3 found in can is 26.3 mg. (0.4 mg. + 25.9 mg..) or percent recovery is 26.3%.
[k] Juice prepared by extraction procedure No. 2.
[l] Pulp prepared by extraction procedure No. 2.
[m] Determination of the Santomerse by method No. 2.

SUMMARY

The data presented shows that satisfactory extraction techniques were developed and that the methods for

detection are sensitive to minute amounts of surface active agents present.

A comparison of the Modified Jones Method with that of Barr, Oliver, and Stubbings indicates that the latter is more readily used and is sufficiently sensitive altho the Jones Method will detect smaller amounts.

By either method, 0.0025% or 25 p.p.m. of dodecylbenzene sodium sulfonate added to, or retained in canned foods may be definitely detected, and larger amounts roughly estimated.

At the time this investigation was undertaken no reports were found in the literature of methods for detecting or determining anionic surface active agents in food products. The authors believe that the methods described in this report may serve as bases for the development of precise quantitative procedures. Improved extraction techniques will undoubtedly lead to better recovery of added surfactant.

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Radiological Contamination of Food

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Atomic or radiological warfare will bring the military forces and civilian population face to face with possible radiological contamination of food. In this report consideration is limited to the contamination of food by radioactive particulate matter. Factors influencing the extent of such contamination and the conditions under which food may be safely salvaged are discussed.

In the event of an attack employing atomic weapons, one of the many problems which would be encountered by both civilian and military authorities is the contamination of food supplies. The following discussion considers the contamination problem arising from the radioactive particulate matter which is widely dispersed by an atomic explosion. Throughout the discussion it is assumed that the level of external radiation [a] is low enough to allow salvage operations to be conducted with safety.[b] No special consideration is given to the alteration of the chemical composition of either food stuffs or packaging materials by induced radioactivity since such activity occurs only in the zone of high neutron flux.

Peterson, Webster, and Liljegren *(4)* indicate that neutron-induced radioactivities in food may be calculated from their sodium, potassium, and phosphorus contents. Furthermore, radioactivity induced in foods at a distance of 1,700 feet from the explosion of a nominal bomb is so low that the ingestion of any reasonable amount is well below accepted tolerance doses.

Since food is one of the basic requirements, the determination of the edibility of supplies in the vicinity of an atomic attack is of prime importance to those responsible for relief and rehabilitation in such areas. While the hazard involved in the ingestion of radioactive material varies with the nature and amounts of these materials, the maximum concentration which can be safely ingested is extremely low. This is especially true of the longer lived alpha and beta emitters present in the fission products of plutonium and uranium as indicated in Tables 1 and 2.

Bale *(1)* considers that levels of beta activity as high as 2×10^5 disintegrations per minute per cubic centi-

TABLE 1

Amounts of radioisotopes considered dangerous (3)

Radioisotopes	Amount (μc)[c]
As^{77}, Kr^{85}	1000
Sr^{89}, Cb^{95}, Ru^{103}, Ru^{106}, Te^{127} Te^{129}, I^{131}, Cs^{137}, Ba^{140}, La^{140} Ce^{141}, Pr^{143}, Nd^{147}	100
Sr^{90}, Y^{91}, Zr^{95}, Ce^{144}, 61^{147}	10

[c] Microcuries.

TABLE 2

One year tolerance values in water (2)

Radioisotope	Amount ($\mu g/c.c.$)[d]
Pr^{239}	3.1×10^{-3}
Sr^{90}	1.3×10^{-7}
Sr^{89}, Y^{90}	4.8×10^{-6}
I^{131}	4.4×10^{-9}
Ba^{140}, La^{140}	5.1×10^{-6}

[d] Micrograms per cubic centimeter.

[a] Definitions of the various units employed in this article may be found in reference *(5)*.

[b] Present AEC tolerance levels for external radiation are 300 milliroentgen (mr.) per week or 7.5 mr. per hour based on a 40-hour week. It seems reasonable, however, that in an emergency a dose of 5 to 25 roentgen (r.) could be condoned, if not repeated.

meter (d. per m. per c.c.) are permissible in water or food in a 10-day period following an atomic bomb blast. Furthermore, he regards levels $\frac{1}{25}$ as great as presenting insignificant risks. Risks associated with alpha activity are negligible in comparison with the beta activity hazards during the first few days after detonation of an atomic bomb. According to Bale, levels of 10^4 alpha d. per m. per c.c. from Pu offer little hazard in water for 10 days.

The extent to which food supplies may be contaminated by radioactivity depends principally upon the following: a, nature of the attack; b, distance between food storage and epicenter of the explosion; and c, type of package.

The nature of the attack may be either a bomb drop or radiological warfare. The bomb drop may be an air burst, an underground one or an underwater one. Since fission products resulting from an air burst are chiefly dissipated in the stratosphere, the only problem of contamination appears in the range of high neutron flux. The underwater and underground bursts are of grave concern since many cities might undergo them. In such bursts, tests have shown that the base surge and fall-out distribute the fission products over wide areas. In the radioactive particulate matter dispersed by such bursts, the contaminants are both long-lived alpha and beta-gamma emitters and also short-lived beta and gamma emitters.

In radiological warfare, radioactive materials are scattered by means other than the explosions of an atomic bomb. Although it is not certain what material would be employed in such warfare, it is reasonable to assume that they would consist of long-lived beta-gamma emitters. Consequently, the problem of food contamination by radiological warfare would be very similar to that encountered in an underwater or underground burst.

The distance of the food supplies from the epicenter of the explosion affects the problem of food contamination in two ways. First, if the storage point is within a certain radius of the epicenter, the blast pressure and heat effects will make the supplies unusable because of physical damage. Secondly, if the storage point is within the range of high neutron flux, materials such as metal containers and chemical preservatives will become radioactive from their bombardment by neutrons. Such induced radioactivity alters the composition of these materials and presents a problem which is distinct from the contamination caused by the widespread diffusion of radioactive particulate matter.

When contamination is caused by radioactive particulate matter, the composition and construction of the package are important factors in preventing contamination of food supplies. Here the problem is essentially the prevention of the passage of radioactive particles from the exterior to the interior of the package. Since these particles may be as small as 0.00002 inch in diameter, the need for an extremely tight package is obvious. Furthermore, the package should be constructed so that its contents can be salvaged without their contamination by contact with radioactive particles on its exterior.

The protection which such common packaging material as sheet metal, metal foil, plastic, paper, fabrics, glass, and wood affords against contamination by radioactive particulate matter merits comment.

Metal, either sheet or foil, is an excellent packaging material. Sheet metal containers are clearly the superior package as far as prevention of dust penetration is concerned. Metal foil affords good protection, but sealing of the seams is a possible weakness.

Plastics are generally good protectors. In the case of bags made of sheet plastic, attention should be directed toward the sealing and the ease with which some of this material is ruptured during rough handling.

Paper containers vary in quality over wide limits. Tight cardboard containers which are covered and well sealed with waxed paper should afford adequate protection while ordinary paper sacks may allow their contents to become contaminated readily. Protection depends on the quality of the material and the care with which the package is sealed.

Such packages as burlap bags and the closer-woven sugar and flour sacks are very porous. These are all obviously poor for preventing contamination of the food supplies contained in them.

Glass generally makes an excellent protective package except for its fragility.

Wood is generally a poor package material unless used in combination with an impermeable material. An exception is the tight, waxed barrel used for many foods.

Although packaging materials for the protection of food from radioactive contaminants are available, techniques must be devised to remove food from containers without contaminating it with radioactive material lodged on their exteriors. Such methods as multiple layer packaging or suitable chemical decontamination are possible ways of solving this problem. Whatever method is used in salvaging food stuffs from contaminated containers, it must be remembered that small quantities of radioactive material may be hazardous if ingested.

The thick peeling which encloses the edible part of a fruit is a protective covering against radioactive contaminants. Fruit like bananas or oranges may be salvaged if care is taken in peeling them. Foods with no protective peeling, such as lettuce, cauliflower, or cabbage, should be discarded.

Standard methods of radiochemical analysis can be easily adapted for determining which foods are safe and which are not. Sampling techniques which allow the evaluation of large quantities in a reasonably short length of time should be employed.

The extent of the contamination found in some food supplies aboard one ship after the Baker underwater explosion during Operation Crossroads is shown in Table 3. Both the condition of the package and the

TABLE 3

Examples of contamination of foodstuffs exposed during Baker test—Operation Crossroads

Food	Condition when found	Estimated fission product activity at 1 minute after blast (mc./g.)*
Canned meat	Can burst open	2.0
Sugar	Open package	0.3
Beans	Sack opened	1.0
Flour	Open barrel	2.5
Brown sugar	Open sack	3.0

* Millicuries per gram.